EVALUATING PULSE-AMPLITUDE MODULATED FLUOROMETRY FOR LANDSCAPE SCALE ASSESSMENT OF PHOTOSYNTHETIC CHARACTERISTICS

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ABSTRACT

Pulse-amplitude modulated fluorometry (PAM) was evaluated for monitoring the physiological condition of the seagrass, *Thalassia testudinum*, in Florida Bay. This approach is attractive because it is rapid, non-invasive, and offers quantitative physiological information. Yet, problems arise when expanding sampling from the organismal scale to the landscape scale, due to temporal changes in photophysiology. The magnitude of diurnal variation of photosynthetic characteristics was investigated using Rapid Light Curves (RLC), which measure quantum yield over a range of changing actinic irradiances. In this study, the resulting calculated parameters (alpha and ETRmax) significantly changed diurnally, as was previously found with effective quantum yield. The significance of among basin and year comparisons was also confounded by diurnal variation and rigorous statistical analysis was unable to discern which time of day was best suited for assessing the photophysiological status of *T. testudinum.* Even though measurements taken with PAM fluorometry exhibited a significant amount of diurnal noise, informative physiological patterns did emerge. The ability to distinguish among basins and see landscape scale trends within the bay gives us an indication that PAM fluorometry may be useful as a monitoring tool. However, in ecosystems where the magnitude of changes are large and occur on much faster timescales than the ecosystem changes that you are trying to measure, the resulting fluctuations may obscure the true physiological signal. Therefore, when using this approach over large spatial and temporal scales diurnal variability must be considered.

In order to incorporate the entire temporal and spatial scale two previously proposed methods, the Diel Yield and Diel Rapid Light Curve method, were investigated. Photosynthesis irradiance (P-E) curves were calculating using both methods and the

ability of each method to accurately predict the relationship between electron transport and irradiance was explored. It was found that neither method was able to provide consistent estimates of photosynthetic efficiency or capacity. The Diel Yield method frequently produced unrealistic predictions of photosynthetic capacity (rETRmax) and saturation irradiance (I_k). The Diel RLC method produced more reasonable predictions of rETRmax and I_k, but this method had the most difficulty predicting photosynthetic efficiency (alpha) when ambient irradiances were continuously high throughout the day ($>I_k$). With some further calculations we believe the Diel RLC method can provide an estimate of photosynthetic efficiency and offer a way to reasonably approximate landscape-level photosynthetic characteristics. Because both methods investigated in this study use data generated from RLCs, which have been shown to vary depending on previous light history; diurnal variations do affect estimates of electron transport rates. Therefore, the Diel RLC method does not negate diurnal variation but it does produce a curve that incorporates the changing ambient light environment.

ACKNOWLEDGMENTS

I would like to thank Dr. Michael J. Durako for his guidance and enthusiasm, which has helped turn me into a scientist. I am so grateful for the opportunity he gave me to explore the realm of photosynthesis and work in an amazing system, Florida Bay. I would also like to thank Dr. Courtney Hackney for helping me believe in myself as a scientist and showing me that it is okay to see the world with child-like intrigue. For the endless hours of statistical guidance, I would like to thank Dr. James Blum. Without him I would never have been able to see the patterns of my data. Field work would have never been possible, and surely not as fun without the help of Manuel Merello, Donna Burns, Dr. Penny Hall, and Brooke Landry. Finally, I would like to thank the biology faculty of the University of North Carolina, Wilmington for making me feel like part of the team.

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CHAPTER 1

DIURNAL VARIATION OF PHOTOSYNTHETIC CHARACTERISTICS MEASURED WITH PULSE-AMPLITUDE MODULATED (PAM) FLUOROMETRY

INTRODUCTION

Pulse-Amplitude Modulated (PAM) fluorometers provide quantitative information about photosystem II (PSII) by measuring chlorophyll fluorescence. PAM fluorometers deliver light of differing intensities and subsequently record the amount of fluorescence re-emitted. First a weak measuring light is applied to determine the minimum fluorescence (F_o dark adapted, F_o' light adapted), which gives a measure of the relative number of open (oxidized) PSII reaction centers. A short saturating light pulse is then applied to determine the maximum fluorescence (F_m dark adapted, F_m' light adapted), resulting from the reduction of all reaction centers (Ralph and Gademann 2005; Maxwell and Johnson 2000). The fluorescence yields can then be used to calculate a variety of photosynthetic coefficients. If the sample is dark adapted, which allows all reaction centers to relax; the maximum quantum yield can be calculated (F_m-F_o /F_m). This provides information about the potential quantum efficiency of PSII (Maxwell and Johnson 2000). Alternatively, if the sample is adapted to the light of its environment, then the effective quantum yield can be calculated (F_m'-F_o '/F_m'). This gives an approximation of the proportion of absorbed energy being used for photochemistry, at a given point in time (Maxwell and Johnson 2000; Genty et al. 1989).

Because PSII is one of the most vulnerable parts of the photosystems it is often the first to manifest the effects of stress (Maxwell and Johnson 2000). Many studies assessing the health of seagrasses have attributed changes in PAM-derived photosynthetic parameters (effective and maximum yields, alpha, and ETRmax) to

environmental stresses. Decreases in quantum yield have been reported in conjunction with exposure to UV radiation (Dawson and Dennison 1996), pathogenic infection (Ralph and Short 2002), herbicide exposure (Ralph and Macinnis 2003), both high (Ralph and Burchett 1995) and low irradiances (Campbell et al. 2003), and an additive combination of stressors (Ralph 1999). Campbell et al. (2003) reported low quantum yields and electron transport rates (ETR) in seagrasses exposed to chronic low-light conditions. They believed that the low photosynthetic quantum yields explained the two- to three-fold lower leaf biomass in plants found in the low light environment. PAM fluorometry is attractive as a monitoring tool because it is rapid, non-destructive, and can provide in-depth, quantitative physiological information about photosynthetic organisms. This information may be used to identify changes in physiological health before morphological or density-based changes are evident.

The Fish Habitat Assessment Program (FHAP) was initiated as part of a multi-agency coalition to assess temporal and spatial changes in macrophyte distribution, abundance and health in the Florida Bay ecosystem (see Durako et al. 2002 for more information on FHAP). Durako and Kunzelman (2002) initially used Pulse Amplitude Modulated (PAM) Fluorometry in FHAP during the spring 2000 sampling to assess the physiological state of *Thalassia testudinum* Banks ex König (Hydrocharitaceae). *T. testudinum* is the dominant macrophyte in the shallow waters of Florida Bay (Zieman 1982) and its abundance is perceived by the public as an indicator of the health of the bay (Durako et al. 2002). FHAP samples ten basins, which represent the range of conditions within Florida Bay; each basin takes an entire day to sample. Although stations are located using a systematic random sample design, to efficiently sample

such a large area, stations must be visited systematically in order to minimize station-to-station travel time. However, Durako and Kunzelman (2002) found that this introduced a significant source of diurnal variation that confounded the assessment of the seagrasses' physiological state. Regressions of effective and maximum quantum yields versus actinic irradiance or time of day both resulted in significant negative slopes.

T. testudinum is a high-light adapted plant, that has the ability to photoacclimate in response to changes in irradiance (Major and Dunton 2002). Because of *T. testudinum's* blade architecture and growth habit, each leaf blade is exposed to a large irradiance gradient throughout its lifetime (Enriquez et al. 2002). Shallow depth, wave focusing, variable weather patterns and tidal and solar oscillations expose the plants to a wide range of irradiances during each diurnal cycle. Given the extreme fluctuations in light reaching the seagrass leaf blade, both efficient photon capture and photoprotective responses are required for growth and survival.

Photosynthesis is dependent on the quantity and quality of light received, but the rate of carbon fixation is not simply proportional to the rate of photon capture (Kirk 1994). The amount of excited electrons that can be funneled through the photosystem is dependent on redox reactions within the electron transport chain. The fluorescence signal detected by PAM fluorometery can provide information on the redox state of the light reaction centers, the light harvesting efficiency and carbon fixation capacity of photosynthesis (MacIntyre et al. 2000). The redox state is dependent on previous reactions thus, as the amount of captured light increases the possibility of being reduced (not able to accept electrons) increases. This results in a greater amount of fluorescence being emitted or an increase in alternative forms of energy dissipation (i.e.,

non-photochemical quenching; White and Critchley 1999; Marshall et al. 2000). In this way fluorescence yield is dependent on the previous light history of the sample. Though this is a direct relationship it is not always proportional. Therefore, interpreting the measurements taken by PAM fluorometers can be difficult, especially when taking numerous measurements throughout a range of actinic intensities. The sensitivity of PAM fluorometry to the plants' previous light history and diurnal fluctuations poses a special problem when sample regimes are expanded to the landscape scale, such as the FHAP sampling of Florida Bay.

This study investigated the use of rapid light curves (RLC) to overcome the landscape scale variations previously detected when measuring effective and maximum quantum yields (Durako and Kunzleman 2002). By measuring effective quantum yields over a range of increasing actinic light intensities, RLC's provide additional information (alpha, rETRmax) about photosynthetic responses to changing light conditions (Ralph and Gademann 2005). We have also broadened our scope of analyses from the station level to the basin level and have incorporated the diurnal cycle into our analysis. Through comparisons of mean photosynthetic parameters among basins and years we were able to establish some applications and functional limitations of using this approach for landscape scale assessment.

Null Hypotheses:

$H_0$1: Mean rapid light curve parameters and effective yield will not significantly differ among morning (8-11), midday (11-2), and afternoon (2-5), within each basin

$H_0$2: Mean rapid light curve parameters (alpha and rETRmax) and effective yield will not significantly differ among basins and years

METHODS

Study site

This study was conducted in Florida Bay (ca. 25°05'N, 81°45'W), a shallow lagoonal estuary at the southern tip of Florida, USA. The bay is characterized by shallow basins (ca. <1m) divided by carbonate mud banks and mangrove islands (Fourqurean and Robblee 1999). Seagrass community development increases in a gradient from the enclosed northern sections to the more open western sections of the bay. This gradient coincides with changing environmental characteristics within the bay. Sediment type and depth change from northeast (shallow fine-grained) to southwest (deeper muddy-sand) (Zieman et al. 1989). Light attenuation is generally greatest in the eastern and south-central regions and lowest in the north-central and western regions (Phlips et al. 1995). Water temperature is more constant throughout the bay, but can show substantial variation due to the shallow depth (Zieman et al. 1982).

The Fish Habitat Assessment Program (FHAP) samples 10 basins (Table 1) that lie within the borders of the Everglades National Park (ENP). The basins were chosen to represent the range of conditions within the bay. Each basin is divided into 27-33 tessellated hexagonal subunits, and one station is randomly chosen within each subunit. This results in 275-330 stations that are randomly sampled throughout the bay (Figure 1).

Photosynthetic Parameters

Photosynthetic characteristics of *Thalassia testudinum* Banks ex König were measured with an underwater fluorometer, Diving PAM (Walz, Germany), in spring of 2002 and 2004, during FHAP sampling. Rapid light curves (RLC) were performed on four

Table 1. List of basins sampled in Florida Bay and their abbreviations.

Abbreviation	Basin
BLK	Blackwater Sound
CAL	Calusa Key
CRN	Crane Key
EAG	Eagle Key
JON	Johnson Key
MAD	Madeira Bay
RAB	Rabbit Key
RKN	Rankin Lake
TWN	Twin Key
WHP	Whipray Bay

haphazardly-chosen short shoots of *T. testudinum*. The short shoots that were chosen were representative of the shoots observed at each station. The middle of the rank 2 blade of each *T. testudinum* short shoot was gently scraped to remove epiphytes before attaching the dark leaf clip (DIVING-LC). The leaf clip held the Diving PAM fiber optic 5mm from the surface of the blade in 2002. This distance was reduced to 2mm in 2004 in order to allow for a reduction in instrument gain and a higher signal to noise ratio. Each rapid light curve exposed the leaf to eight incremental steps of irradiance ranging from 0 to 2060 µmol photons $m^{-2}s^{-1}$ in 2002, and 0 to 1735 µmol photons $m^{-2}s^{-1}$ in 2004. The reduction in irradiance levels in 2004 was due to slight damage to the fiber optic causing a decrease in light transmission at the same instrument settings. An effective yield measurement (F_m'-F_o'/F_m') was taken at the beginning of each curve, before actinic light was applied, and at the end of each 5s irradiance step, resulting in nine yield measurements for each rapid light curve. Each yield measurement was used to calculate the relative electron transport rate (rETR) through photosystem II using the following equation: rETR=Yield x PAR x AF x 0.5, where PAR is the actinic light generated by the internal halogen lamp of the Diving PAM, AF is the fraction of light absorbed by the leaf, and 0.5 assumes that the photons absorbed are equally partitioned between PSII and PSI (Genty et al. 1989). Note that this is only a relative rate of electron transport since the default AF value of 0.84, set for terrestrial plants, was used in this study.

Photosynthesis-irradiance (P-E) curves were generated from the calculated rETRs and the irradiances applied during the rapid light curve steps. P-E curves have distinct regions that provide useful information about the thermodynamic and metabolic

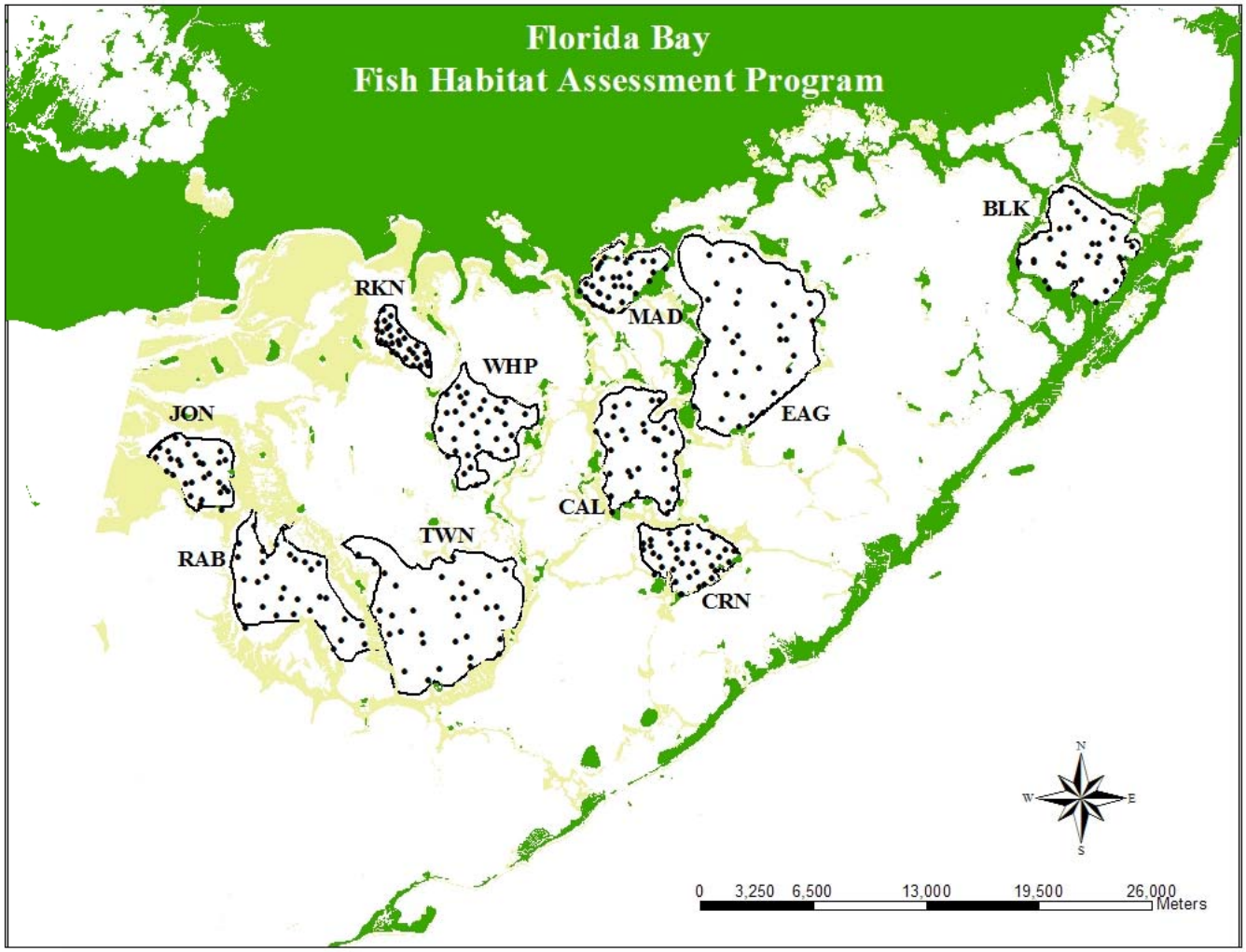


Figure 1. Map of basins and sample stations within Florida Bay

constraints of photosynthesis (Walker 1987). The initial slope of the curve (alpha) is a measure of the light harvesting efficiency of photosynthesis. The asymptote of the curve, the maximum rate of photosynthesis ($rETR_{max}$), is a measure of the capacity of the photosystems to utilize the absorbed light energy (Marshall et al. 2000). These parameters indicate the plants' present and potential photosynthetic capacity (Ralph and Gademann 2005). Rapid light curves are different from traditional P-E curves because each step of illumination does not provide sufficient time for photosynthesis to reach steady state (Schreiber et al.1997). However, rapid light curves can be used to illustrate the acclimation of the photosynthetic apparatus to a range of light intensities, and also provide a reliable assessment of photosynthetic activity (Ralph and Gademann 2005). Each P-E curve was fitted to a double exponential decay function in order to quantify the characteristic parameters (alpha, $rETR_{max}$) (Platt et al. 1980). All curves were generated in SAS (SAS 8.2, Cary, NC) using the NONLIN procedure. The use of rapid light curves enabled us to compare the spatial and temporal variability of effective quantum yield (the first yield measurement taken before light is applied) and the quantified parameters of the P-E curves (alpha and rETRmax).

Temperature, salinity, water depth, and irradiance at the seagrass canopy (PAR_{canopy}) were measured at each station. Ambient irradiance at the seagrass canopy was determined using a quantum PAR scalar sensor (LiCor LI-193S). Salinity and temperature were measured with a handheld digital meter (YSI 30).

Analysis

To assess the effect of diurnal fluctuations during each basin sampling period, within-basin variation of effective yield, RLC parameters (alpha, $rETR_{max,}$), and physical

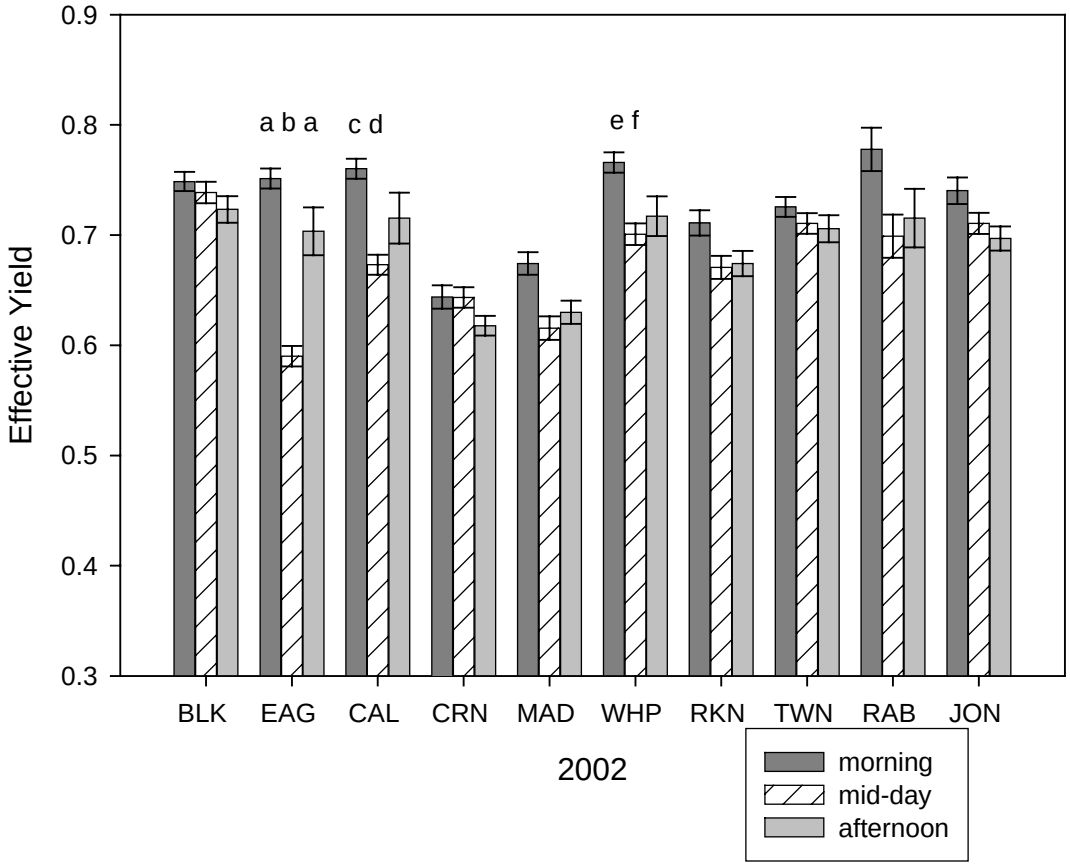
parameters (temperature, PAR_{canopy}) were compared. Measurements of photosynthetic and physical parameters were grouped by morning (8-11), mid-day (11-2) and afternoon (2-5), and means for these three time periods were compared within each basin. To assess variability in photosynthetic parameters among basins and years, mean effective yields and RLC parameters (alpha and $rETR_{max}$) for the three time periods were also compared among basins and years (2002, 2004). Only means of similar time periods were compared. Daily mean parameters were also compared in order to incorporate the entire basin-level spatial scale into our analysis and to contrast with separate time period comparisons. All comparisons were made by multi-factor ANOVA with a Tukey Kramer adjustment for pairwise comparison. Also, Pearson correlations were done in order to examine possible relationships between photosynthetic and physical parameters.

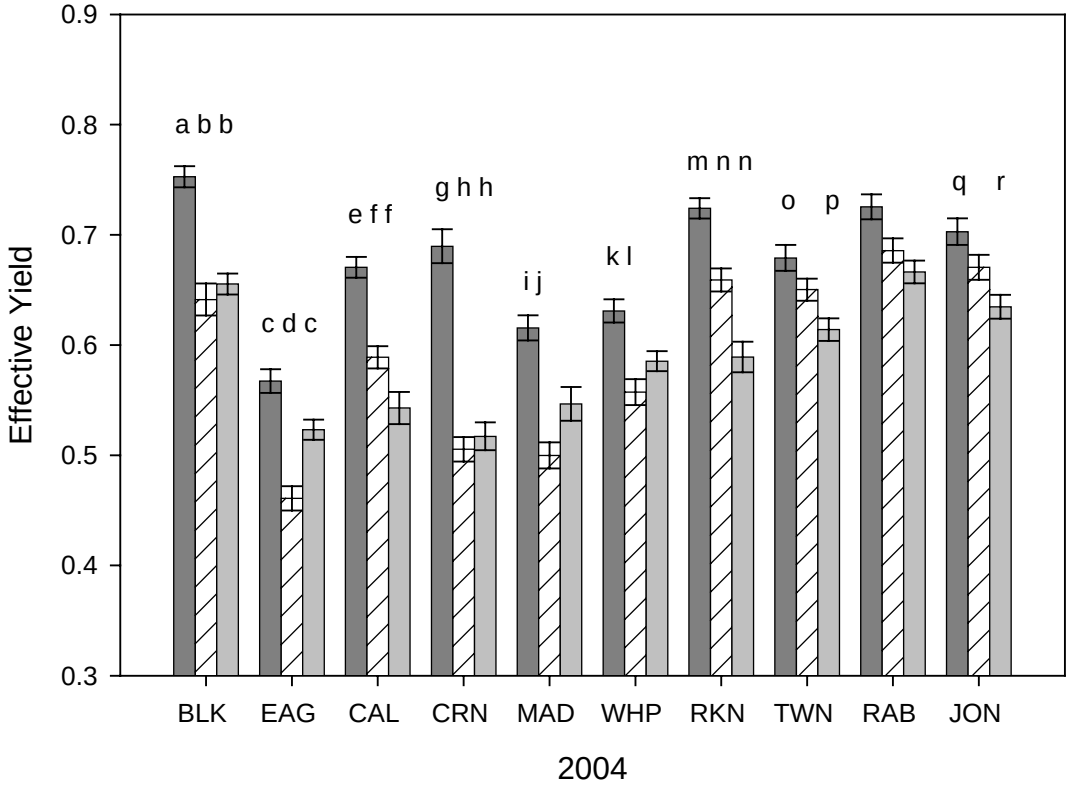
RESULTS

Diurnal Variation of Photosynthetic Parameters

Effective yield (F_m'-F_o'/F_m') showed relatively consistent diurnal patterns in all ten basins and between years (Figure 2). Effective yield was always highest in the morning (8-11) and decreased during the mid-day (11-2). Yields then either exhibited a slight recovery in the afternoon (2-5) or continued to decrease in the afternoon. Though there was a consistent diurnal pattern in all of the basins, the differences among time periods were not always significant and differences in the number of basins that showed a significant diurnal variation also varied between years. Only three basins in 2002 had significant differences among time periods, while in 2004 all but one basin showed significant diurnal variation. Photosynthetic efficiency (alpha) also displayed a diurnal pattern

Figure 2. Comparison of mean morning (8-11), mid-day (11-2), and afternoon (2-5) effective yield (F_m'-F_o'/F_m') of *Thalassia testudinum* blades measured in the ten basins sampled during spring FHAP 2002 and 2004. Basins arranged graphically in conjunction with their spatial position with in the bay (east to west). Letters denoting significant differences among time periods ($p<0.05$, Tukey's post hoc test).

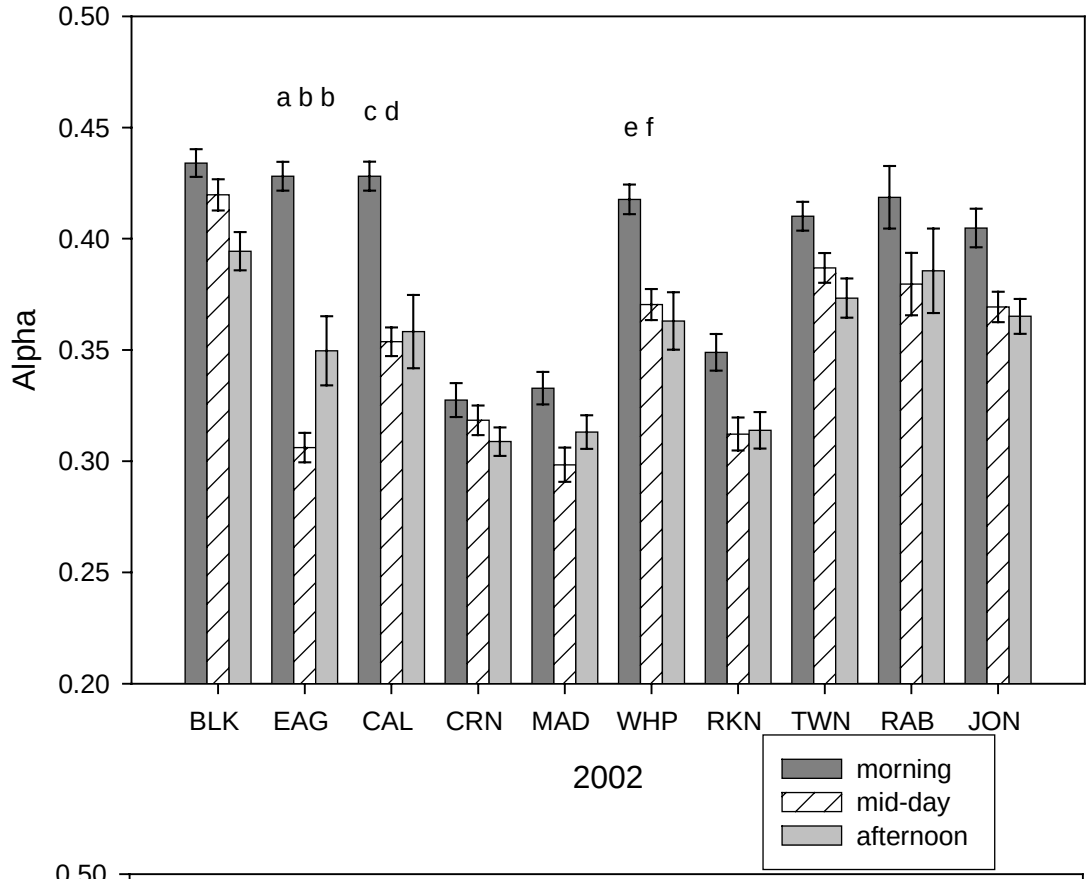

Effective Yield
0.9
0.8
0.7
0.6
0.5
0.4
0.3
a b a
c d
e f
BLK
EAG
CAL
CRN
MAD
WHP
RKN
TWN
RAB
JON
2002
morning
mid-day
afternoon


Effective Yield
0.9
0.8
0.7
0.6
0.5
0.4
0.3
a b b
c d c
e f f
g h h
i j
k l
m n n
o
p
q
r
BLK
EAG
CAL
CRN
MAD
WHP
RKN
TWN
RAB
JON
2004

(Figure 3). Alpha exhibited a pattern very similar to effective quantum yield, with the highest values in the morning, a decrease in efficiency during mid-day, with either a slight recovery in the afternoon or a continued decrease. One exception to this pattern was Eagle Key (EAG) in 2004, which showed an afternoon recovery that was greater than the morning; however, the difference was not significant. Even though all basins illustrated a similar pattern of fluctuation throughout the day, not all basins showed significant differences among time periods. Also, as was found in effective quantum yield, the number of basins that had significant variation among time periods increased from three basins in 2002 to nine basins in 2004. Both effective quantum yield (-0.63 $p < 0.001$ df=1054) and alpha (-0.57 $p < 0.001$ df=1054) were found to be negatively correlated with the irradiance measured at the seagrass canopy (PAR_{canopy} µmol m^{-2} s^{-1}) (Figure 4). While PAR_{canopy} in most basins exhibited a typical diurnal pattern with low irradiance in the morning, highest irradiance around mid-day and a decrease in irradiance as the day progressed, the differences among the time periods we sampled were not always significant. There was a marked increase in irradiance reaching the seagrass canopy in 2004 in most basins (though there was a 20% decrease in irradiance in JON, and RAB and RKN mean irradiance did not change between years, all other basins measured had a 14-161% increase in irradiance) and an increase in the number of basins that exhibited a significant variation in PAR_{canopy} throughout the day, which in turn may be responsible for the increase in diurnal variation seen in both of the photosynthetic parameters in 2004.

Photosynthetic capacity (rETRmax) exhibited a generally consistent but different diurnal pattern compared to effective quantum yield or alpha. rETRmax was lowest in the

Figure 3. Comparison of mean morning (8-11), mid-day (11-2), and afternoon (2-5) rapid light curve generated efficiency (alpha) (µmol e^- $m^{-2}s^{-1}$/µmol photon $m^{-2}s^{-1}$) of *Thalassia testudinum* blades measured in the ten basins sampled during spring FHAP 2002 and 2004. Basins arranged graphically in conjunction with their spatial position with in the bay (east to west). Letters denoting significant differences among time periods ($p<0.05$, Tukey's post hoc test).


0.50
0.45
0.40
0.35
0.30
0.25
0.20
Alpha
a b b
c d
e f
BLK
EAG
CAL
CRN
MAD
WHP
RKN
TWN
RAB
JON
2002
morning
mid-day
afternoon

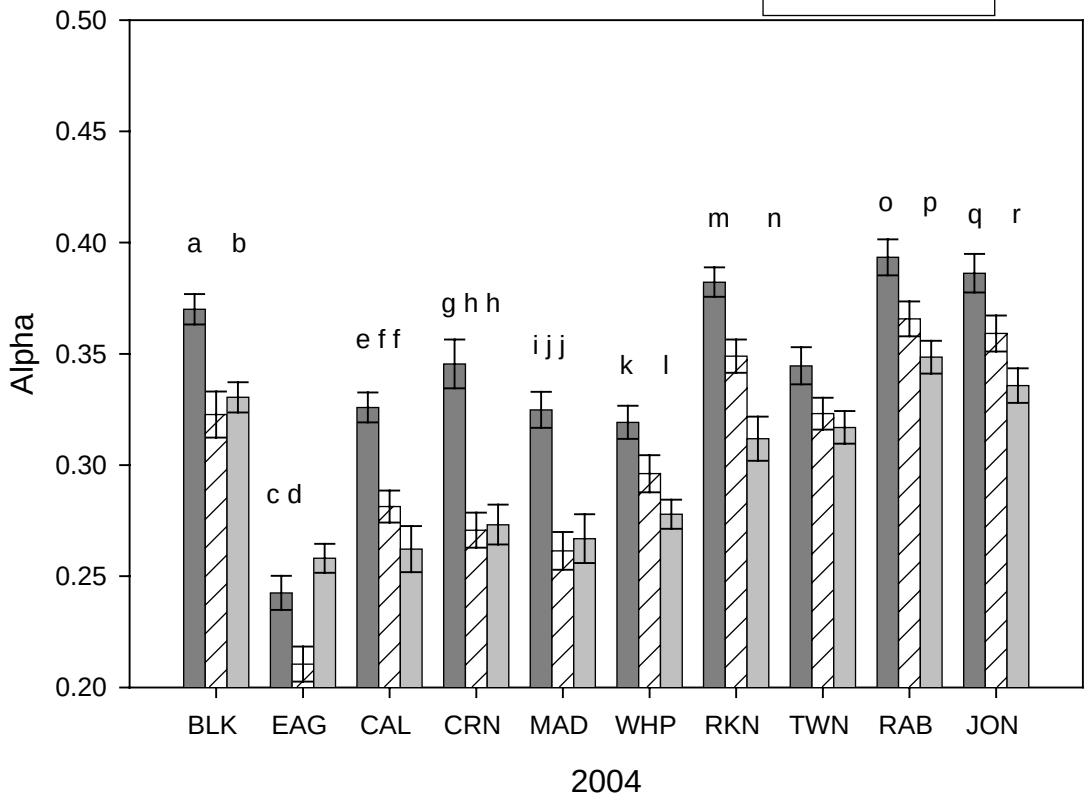
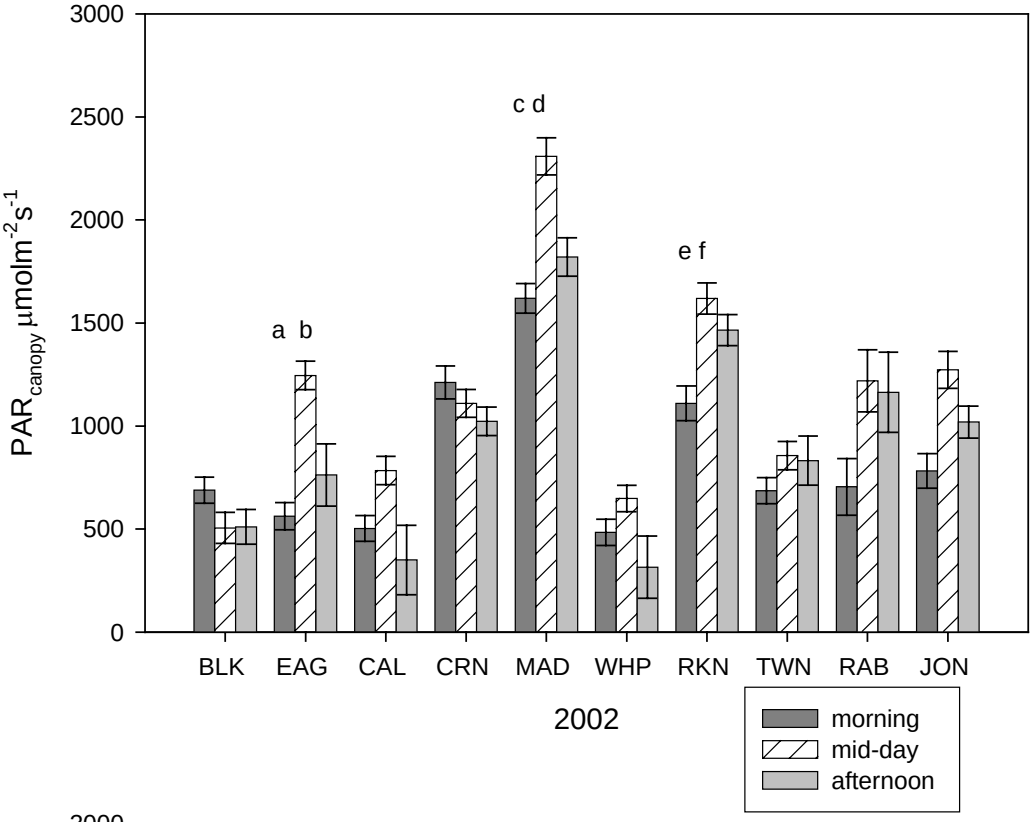
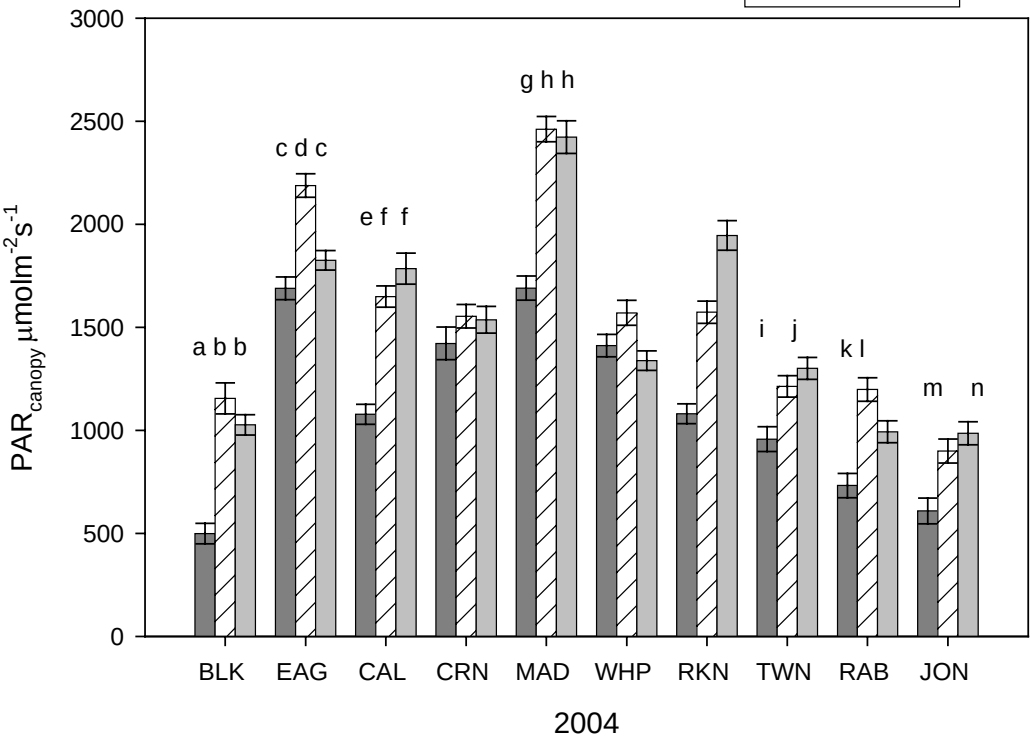

0.50
0.45
0.40
0.35
0.30
0.25
0.20
Alpha
a b
c d
e f f
g h h
i j j
k l
m n
o p
q r
BLK
EAG
CAL
CRN
MAD
WHP
RKN
TWN
RAB
JON
2004

Figure 4. Comparison of mean morning (8-11), mid-day (11-2), and afternoon (2-5) irradiance (µmol photon $m^{-2}s^{-1}$) measured at the seagrass canopy (PAR_{canopy}) in the ten basins sampled during spring FHAP 2002 and 2004. Basins arranged graphically in conjunction with their spatial position with in the bay (east to west). Letters denoting significant differences among time periods ($p<0.05$, Tukey's post hoc test).


3000
2500
2000
1500
1000
500
0
PAR canopy µmolm-2s-1
a b
c d
e f
BLK
EAG
CAL
CRN
MAD
WHP
RKN
TWN
RAB
JON
2002
morning
mid-day
afternoon


3000
2500
2000
1500
1000
500
0
PAR canopy µmolm-2s-1
a b b
c d c
e f f
g h h
i j
k l
m n
BLK
EAG
CAL
CRN
MAD
WHP
RKN
TWN
RAB
JON
2004

morning, and progressively increased through mid-day and afternoon (Figure 5). There were a few exceptions to this general trend; both Crane Key (CRN) in 2002 and Eagle Key (EAG) in 2004 exhibited a slight decline between morning and mid-day. Also CRN in 2004 showed a decline from morning through the afternoon. However, the differences among time periods were not significant. Though rETRmax showed a consistent pattern in most basins, the difference among time periods was not always significant and the

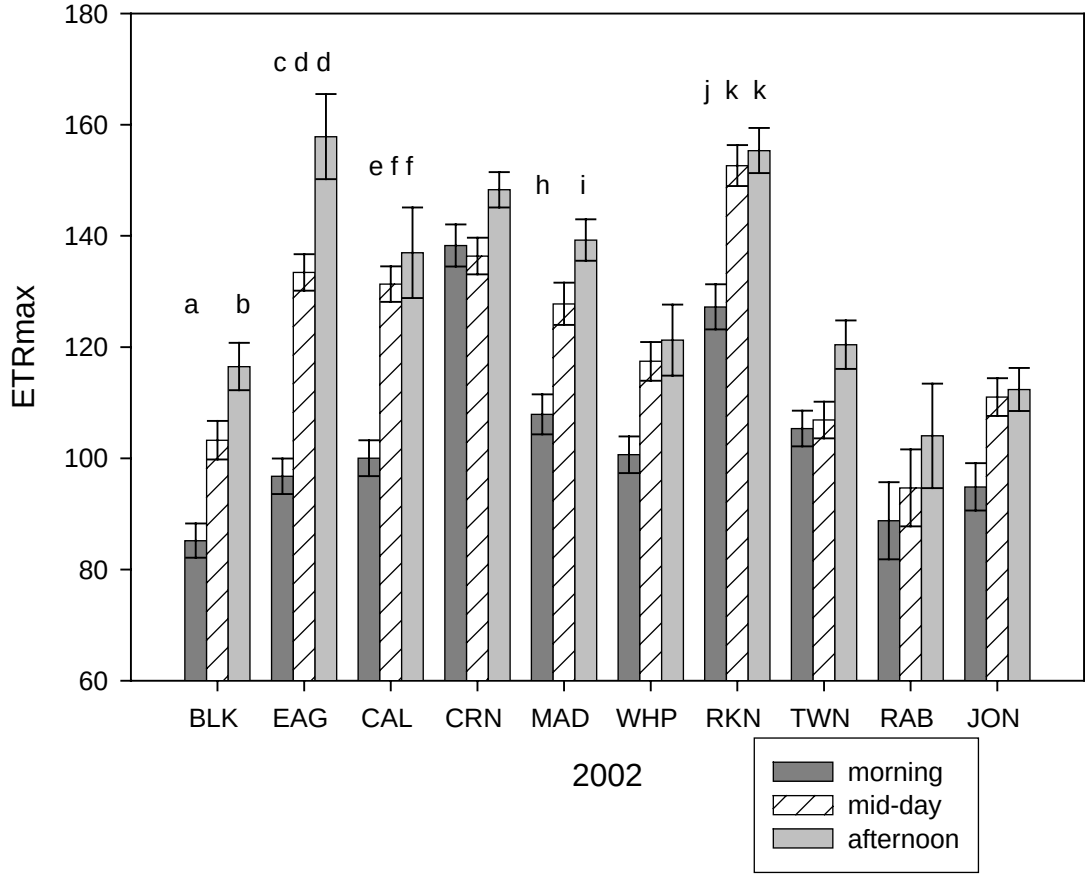
basins that showed significant differences changed between the years. rETRmax was found to be most closely correlated with temperature (0.49 $p<0.0001$) which showed a very similar diurnal trend of increasing throughout the day (Figure 6).

Among-Basin and Between-Year Comparisons

Variability in effective yields (F_m'-F_o'/F_m') among basins differed among time periods and years (Figure 7). Among-basin differences in 2002, as determined by the number of significant Tukey post hoc differences, were greatest during mid-day, lowest in afternoon and intermediate in morning. In 2004, among-basin differences were greatest in the afternoon, lowest in the morning, and showed intermediate variability during mid-day. Interannual variability was also distinct among time periods, with the greatest difference between years occurring during afternoon and the least during the morning.

Mean basin photosynthetic efficiency (alpha) also differed among time periods and years (Figure 8). In 2002, the greatest number of among-basin differences occurred during mid-day, with less variation in morning and afternoon. Mid-day in 2004 also exhibited the greatest amount of among-basin variation, with morning displaying the

Figure 5. Comparison of mean morning (8-11), mid-day (11-2), and afternoon (2-5) photosynthetic capacity ($_rETRmax$) (µmol $e^- m^{-2}s^{-1}$) of *Thalassia testudinum* blades measured in the ten basins sampled during spring FHAP 2002 and 2004. Basins arranged graphically in conjunction with their spatial position with in the bay (east to west). Letters denoting significant differences among time periods ($p<0.05$, Tukey's post hoc test).


ETRmax
180
160
140
120
100
80
60
a
b
c d d
e f f
h
i
j k k
BLK
EAG
CAL
CRN
MAD
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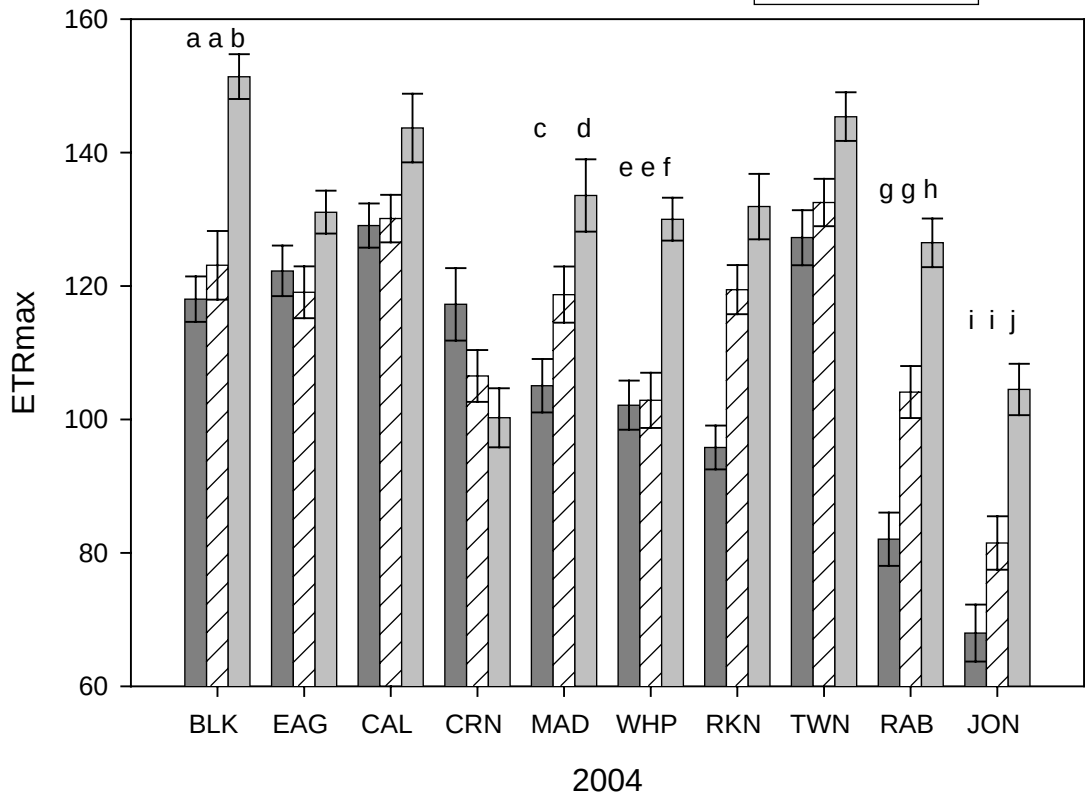
RKN
TWN
RAB
JON
2002
morning
mid-day
afternoon


ETRmax
160
140
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80
60
a a b
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e e f
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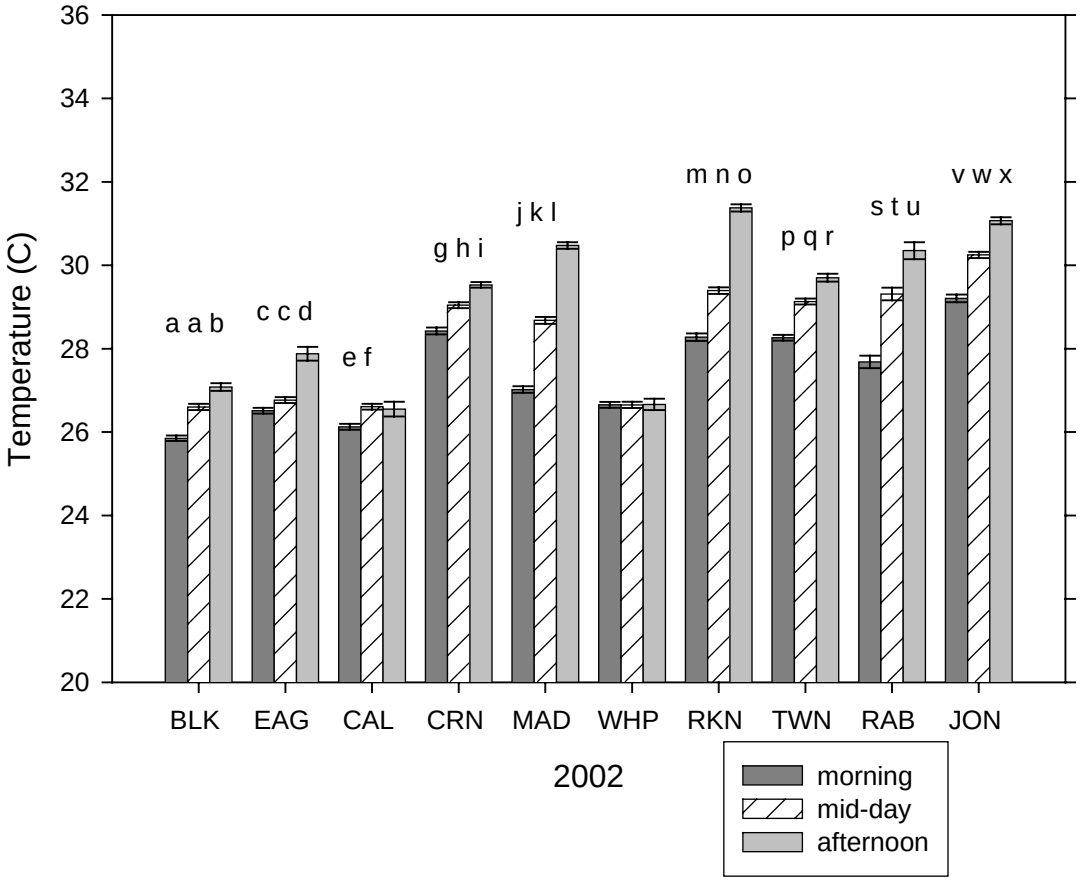
BLK
EAG
CAL
CRN
MAD
WHP
RKN
TWN
RAB
JON
2004

Figure 6. Comparison of mean morning (8-11), mid-day (11-2), and afternoon (2-5) water temperature (˚C) measured in the ten basins sampled during spring FHAP 2002 and 2004. Basins arranged graphically in conjunction with their spatial position with in the bay (east to west). Letters denoting significant differences among time periods ($p<0.05$, Tukey's post hoc test).


Temperature (C)
36
34
32
30
28
26
24
22
20
a a b
c c d
e f
g h i
j k l
m n o
p q r
s t u
v w x
BLK
EAG
CAL
CRN
MAD
WHP
RKN
TWN
RAB
JON
2002
morning
mid-day
afternoon

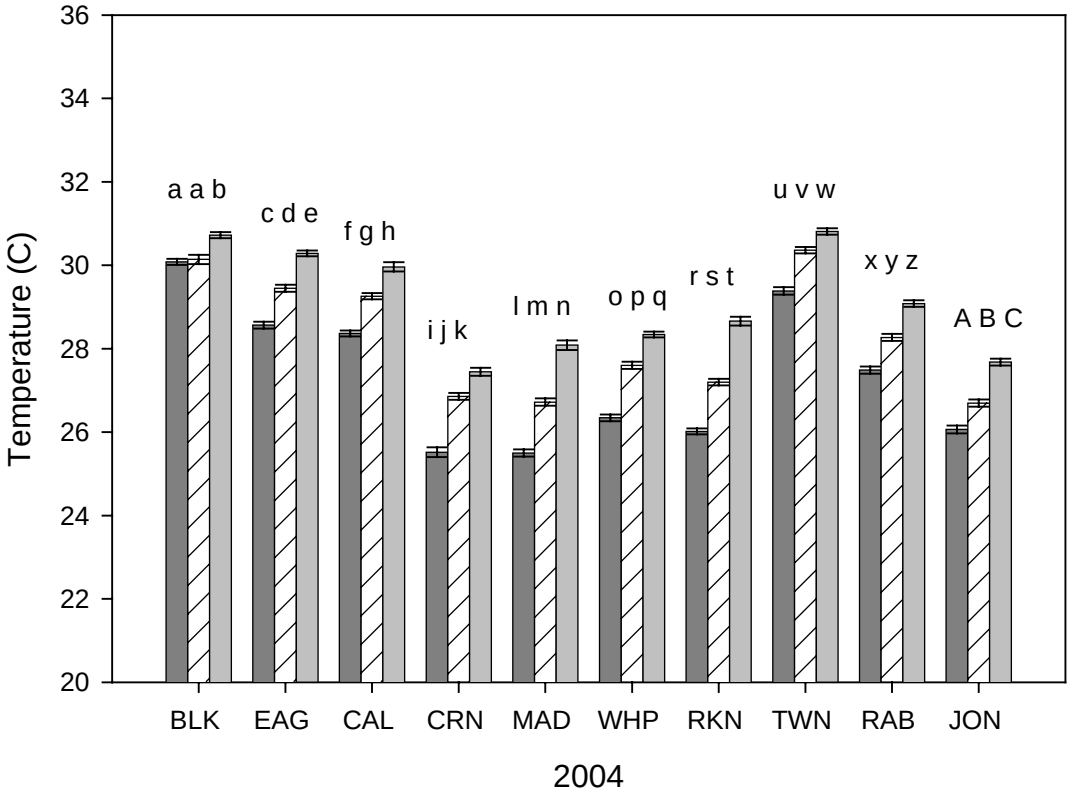

Temperature (C)
36
34
32
30
28
26
24
22
20
a a b
c d e
f g h
i j k
l m n
o p q
r s t
u v w
x y z
A B C
BLK
EAG
CAL
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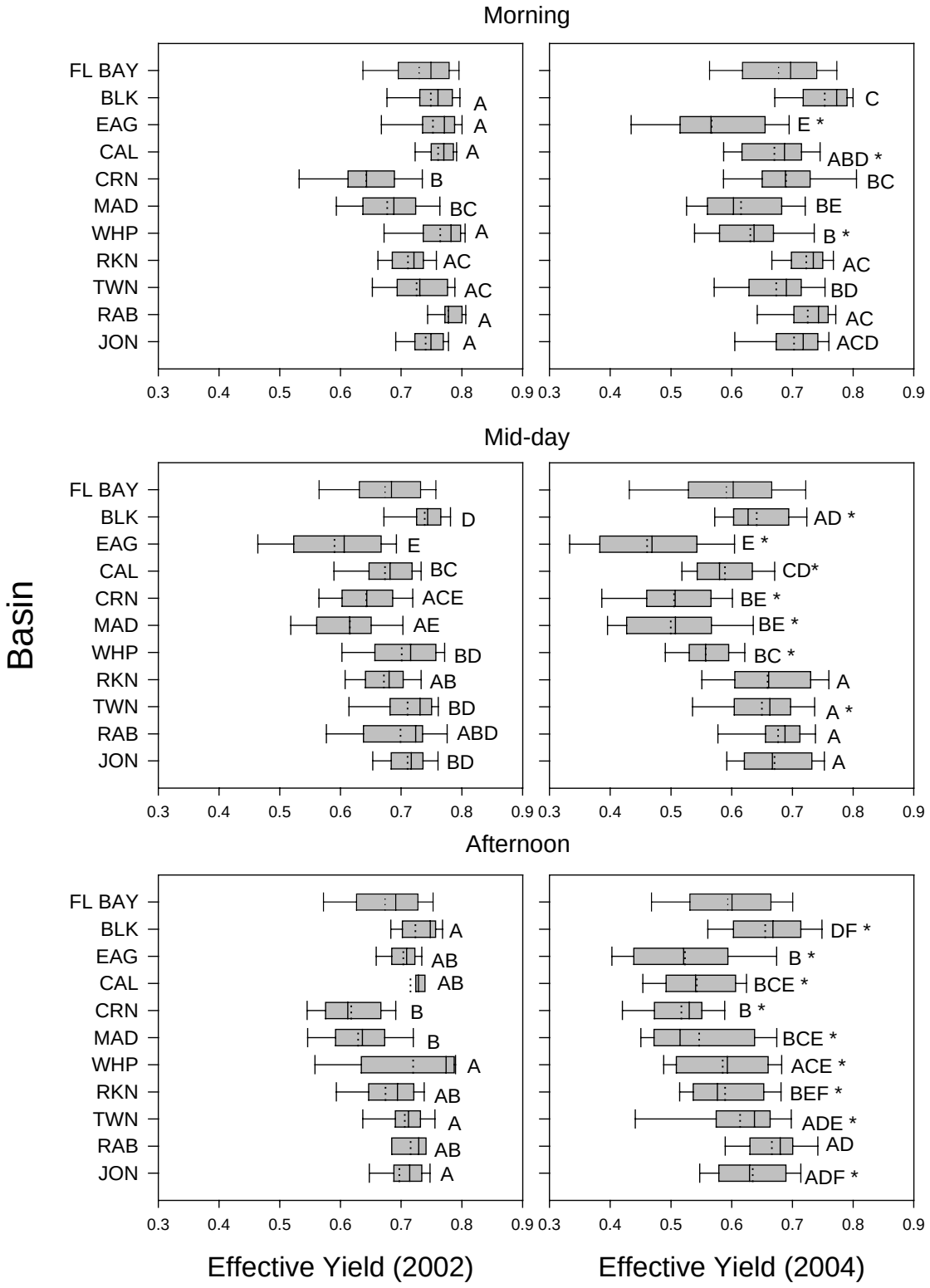
Figure 7. Effective yield (F_m'-F_o '/F_m') comparisons among the 10 basins sampled in Florida Bay measured within similar time periods in 2002 and 2004. Basins with different letters are significantly different within a time period and basins marked with an asterisk (*) exhibited significant differences between years during the specific time period ($p<0.05$, Tukey's post hoc test). Basins arranged graphically in conjunction with their spatial position with in the bay (east to west). Box and whisker diagrams: boxes enclose the 25^{th} and 75^{th} percentile, whiskers enclose the 10^{th} and 90^{th} percentile, vertical line within box represents median, and dashed vertical line represents the mean. Values for the entire Bay during each time period are shown for comparison.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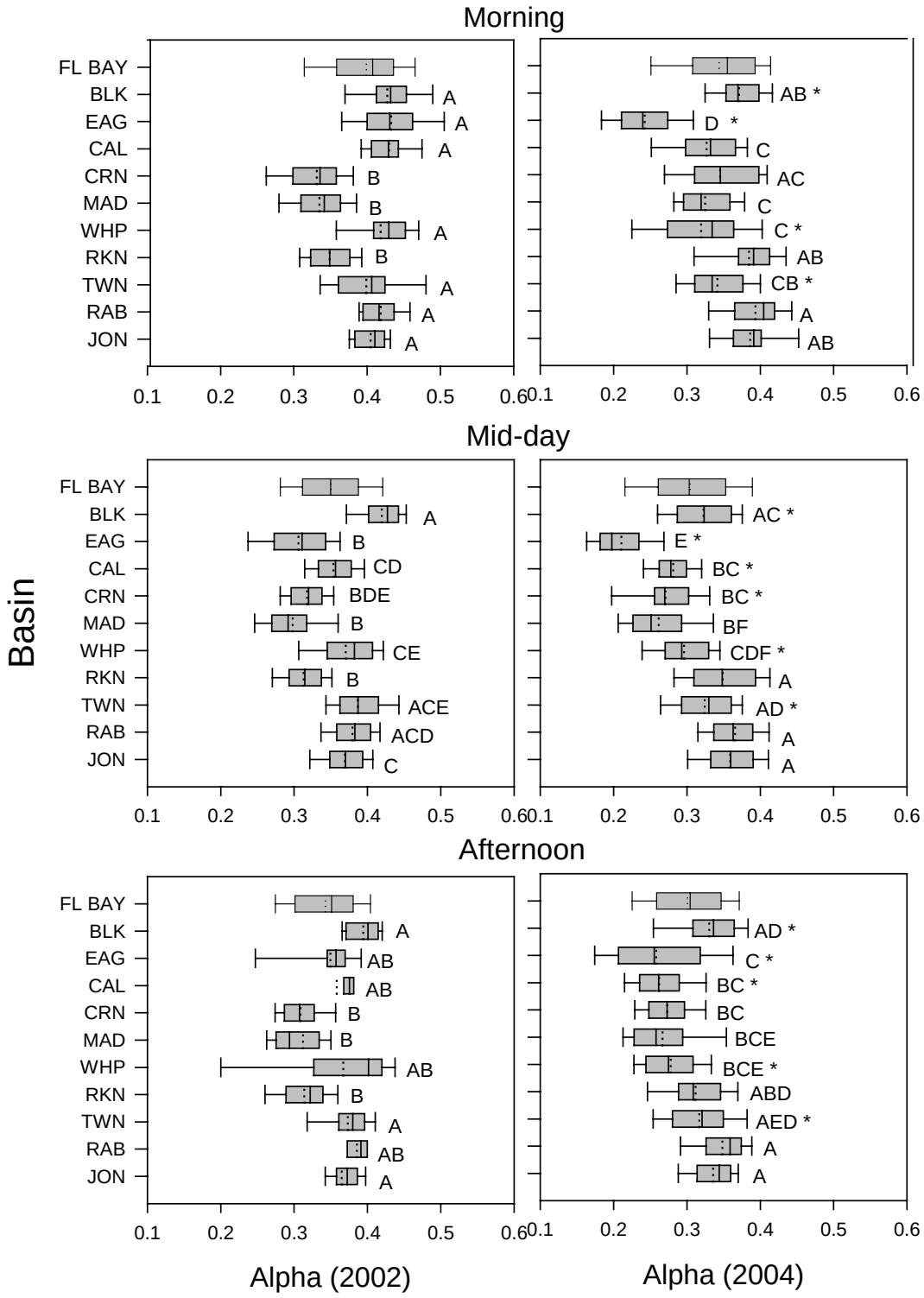
Effective Yield (2002)
Effective Yield (2004)

Figure 8. Photosynthetic efficiency (alpha) (µmol e^{-} $m^{-2}s^{-1}$/µmol photon $m^{-2}s^{-1}$) comparisons among the 10 basins sampled in Florida Bay measured within similar time periods in 2002 and 2004. Basins with different letters are significantly different within a time period and basins marked with an asterisk (*) exhibited significant differences between years during the specific time period ($p<0.05$, Tukey's post hoc test). Basins arranged graphically in conjunction with their spatial position with in the bay (east to west). Box and whisker diagrams: boxes enclose the 25^{th} and 75^{th} percentile, whiskers enclose the 10^{th} and 90^{th} percentile, vertical line within box represents median, and dashed vertical line represents the mean. Values for the entire Bay during each time period are shown for comparison.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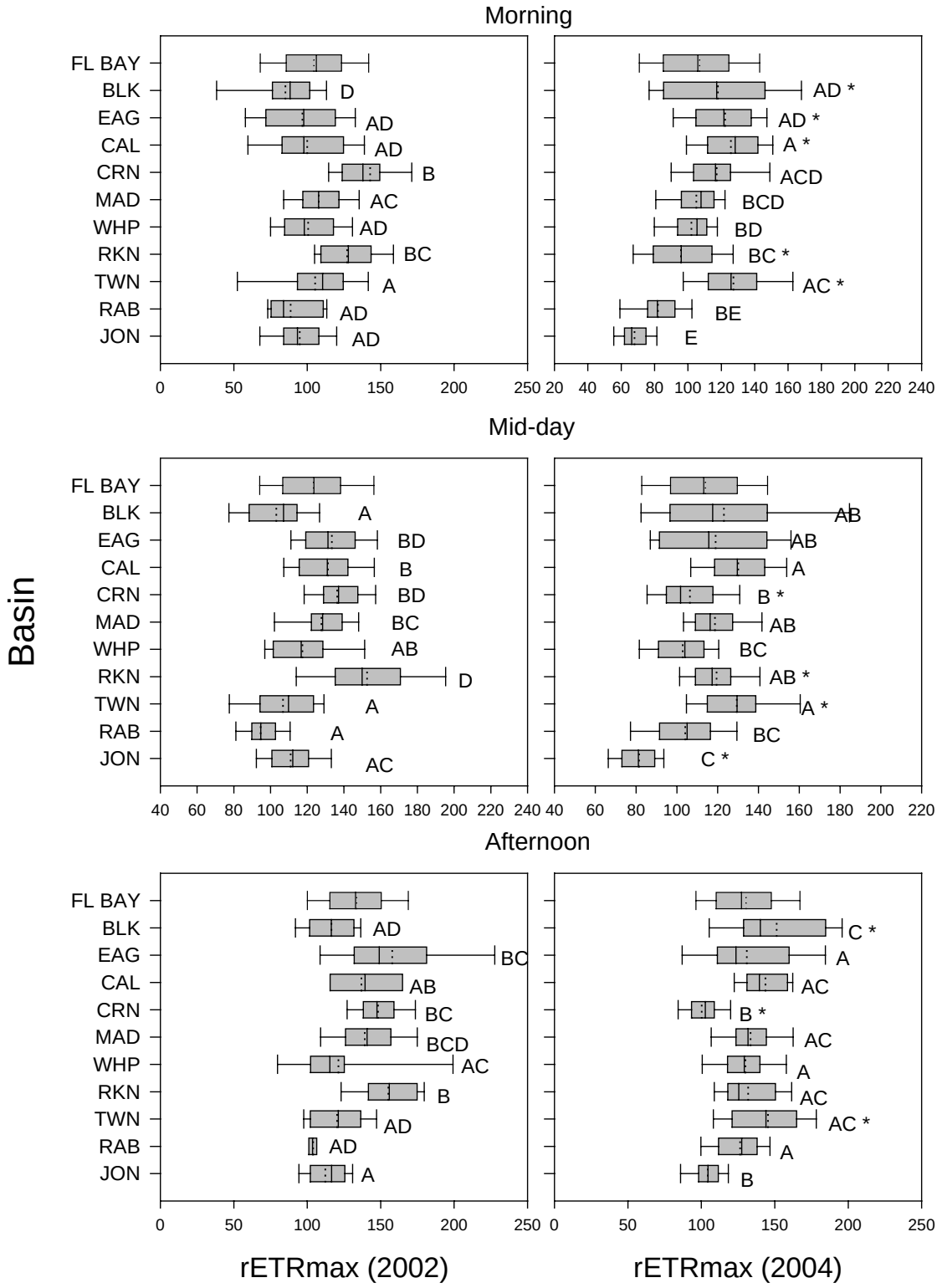
least, and afternoon showing intermediate variability. Interannual variation exhibited incongruity among time periods, with the highest number of significant differences detected during mid-day and the lowest in the morning.

Among-basin variability in photosynthetic capacity (rETRmax) exhibited similar heterogeneity among time periods as the other PAM derived parameters (Figure 9). Among-basin differences were the same during all three time periods in 2002, while morning exhibited the highest significant among-basin variability in 2004, with lower variation occurring in both mid-day and afternoon. The greatest number of within-basin significant differences between years was detected during the morning, with a decrease in the number of significant changes as the day progressed.

Comparisons of the mean daily basin parameters were done in order to elucidate how basins differ if the time of day variation was pooled and the entire spatial scale for each basin was incorporated into the analyses. There was a larger range of mean daily effective yields at the basin and bay scales in 2004 but both years exhibited a similar pattern for among-basin differences (Figure 10). When daily mean effective yields of both years were compared to the three daily time periods, it was found that the daily mean yields exhibited similar among-basin difference patterns as mid-day comparisons during 2002 and afternoon comparisons in 2004 (compare Figures 10 and 7). When comparing daily mean alpha between years, 2002 exhibited a greater number of among basin differences, but 2004 had a greater range (Figure 11). Mean daily alpha in 2002 exhibited more significant variability among basins than any single time period, but 2004 showed less variability than any single time period (compare Figures 11 and 8). When comparing mean daily rETRmax between years, 2002 showed the greatest among

Figure 9. Photosynthetic capacity (rETRmax) (µmol e^{-} $m^{-2}s^{-1}$) comparisons among the 10 basins sampled in Florida Bay measured within similar time periods in 2002 and 2004. Basins with different letters are significantly different within a time period and basins marked with an asterisk (*) exhibited significant differences between years during the specific time period ($p<0.05$, Tukey's post hoc test). Basins arranged graphically in conjunction with their spatial position with in the bay (east to west). Box and whisker diagrams: boxes enclose the 25^{th} and 75^{th} percentile, whiskers enclose the 10^{th} and 90^{th} percentile, vertical line within box represents median, and dashed vertical line represents the mean. Values for the entire Bay during each time period are shown for comparison.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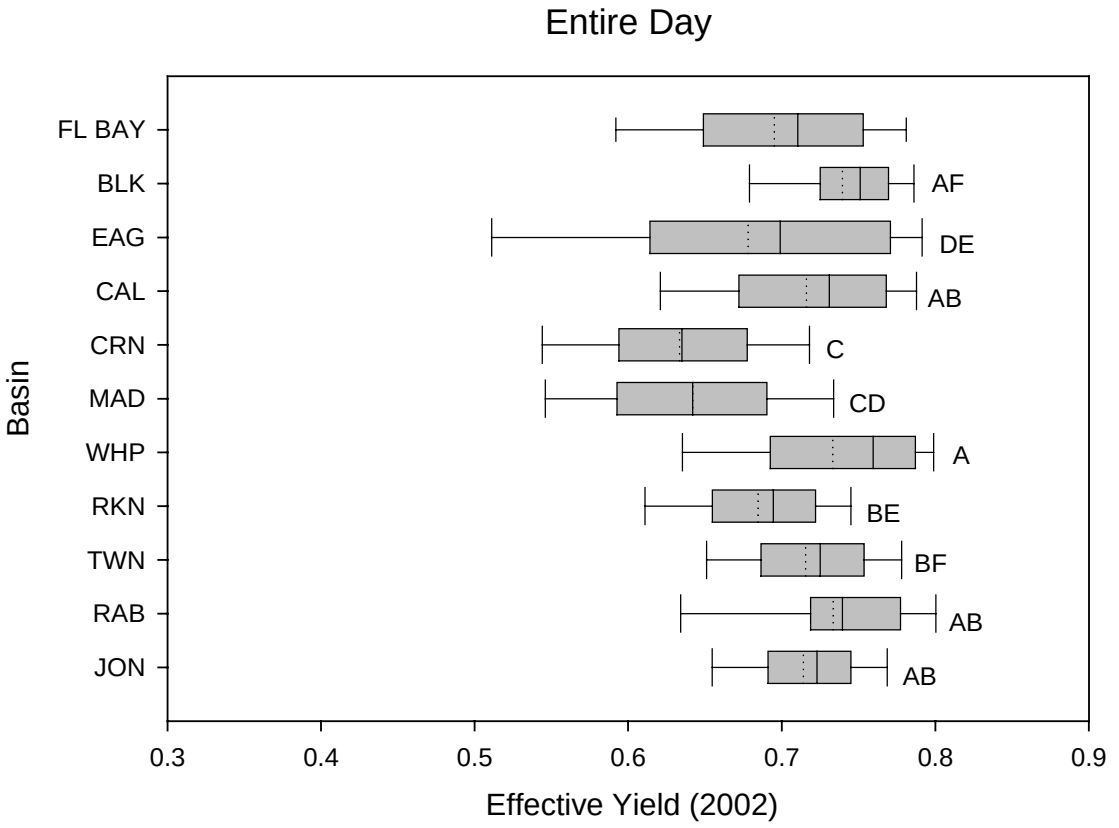
rETRmax (2002)
rETRmax (2004)

Figure 10. Effective yield (F_m'-F_o'/F_m') comparisons among the 10 basins sampled in Florida Bay measured throughout the entire day in 2002 and 2004. Basins with different letters are significantly different and basins marked with an asterisk (*) exhibited significant differences between years ($p<0.05$, Tukey's post hoc test). Basins arranged graphically in conjunction with their spatial position with in the bay (east to west). Box and whisker diagrams: boxes enclose the 25th and 75th percentile, whiskers enclose the 10th and 90th percentile, vertical line within box represents median, and dashed vertical line represents the mean. Values for the entire Bay are shown for comparison.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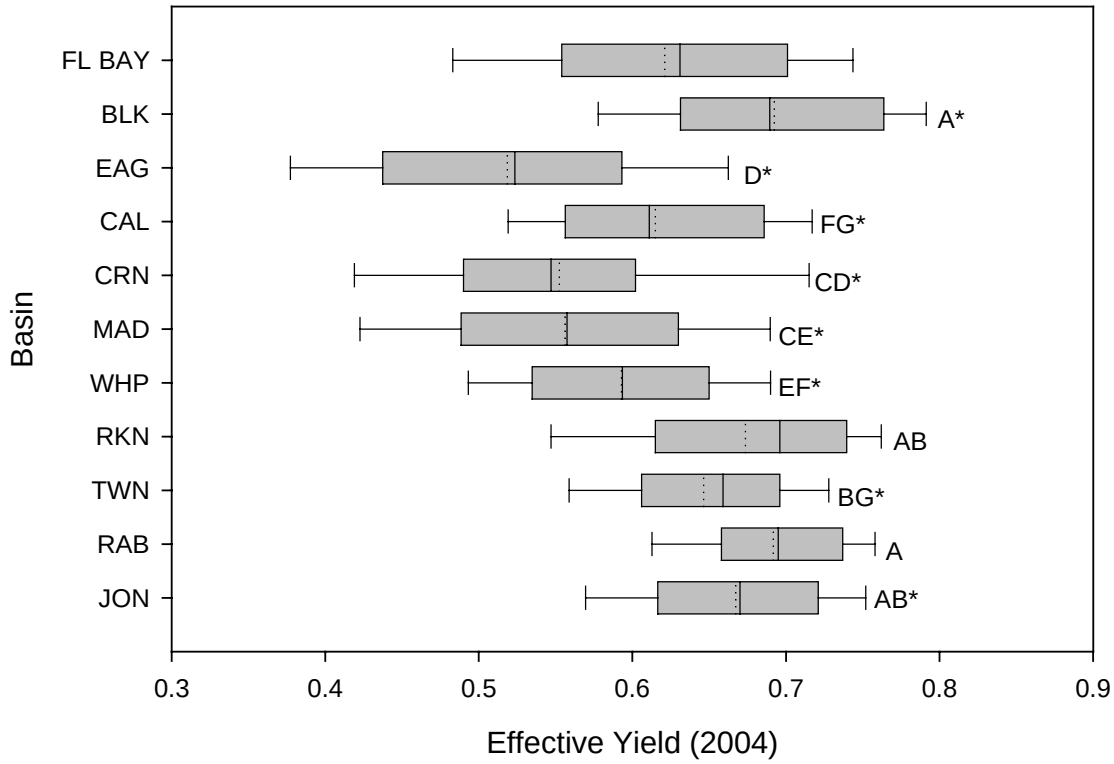
Effective Yield (2002)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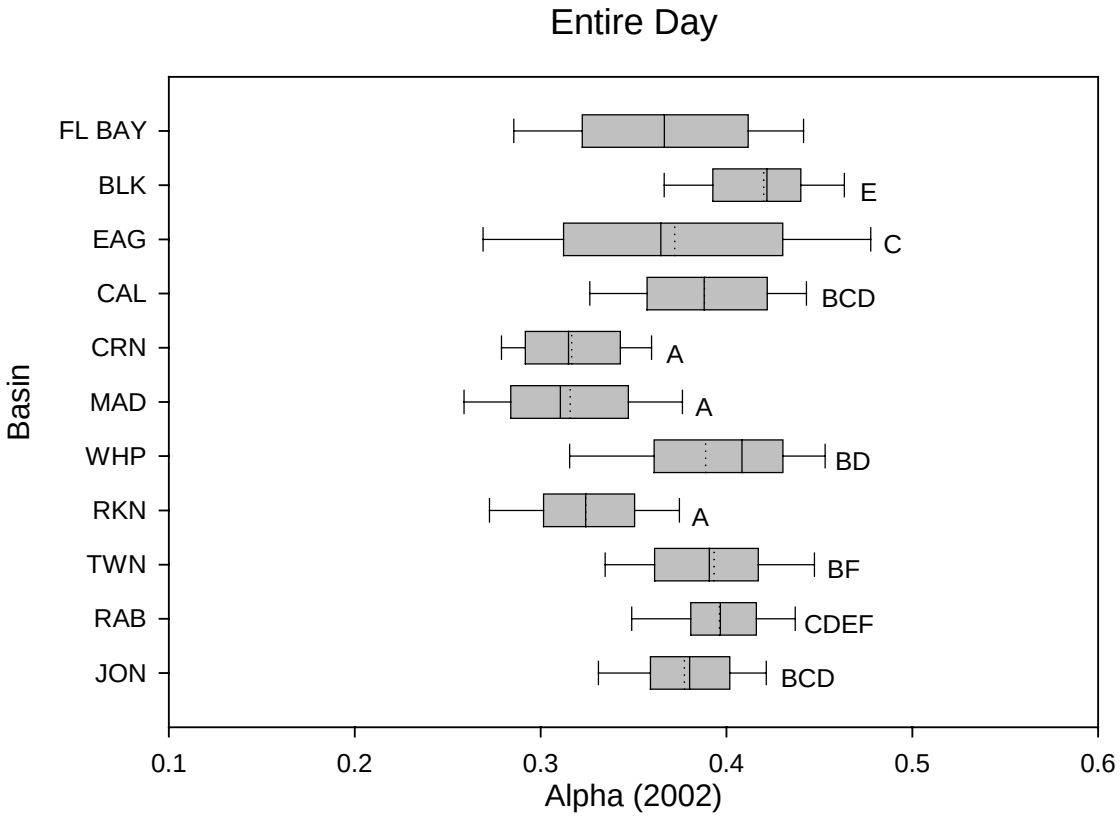
Effective Yield (2004)

Figure 11. Photosynthetic efficiency (alpha) (µmol e^- $m^{-2}s^{-1}$/µmol photon $m^{-2}s^{-1}$) comparisons among the 10 basins sampled in Florida Bay measured throughout the entire day in 2002 and 2004. Basins with different letters are significantly different and basins marked with an asterisk (*) exhibited significant differences between years ($p<0.05$, Tukey's post hoc test). Basins arranged graphically in conjunction with their spatial position with in the bay (east to west). Box and whisker diagrams: boxes enclose the 25^{th} and 75^{th} percentile, whiskers enclose the 10^{th} and 90^{th} percentile, vertical line within box represents median, and dashed vertical line represents the mean. Values for the entire Bay are shown for comparison.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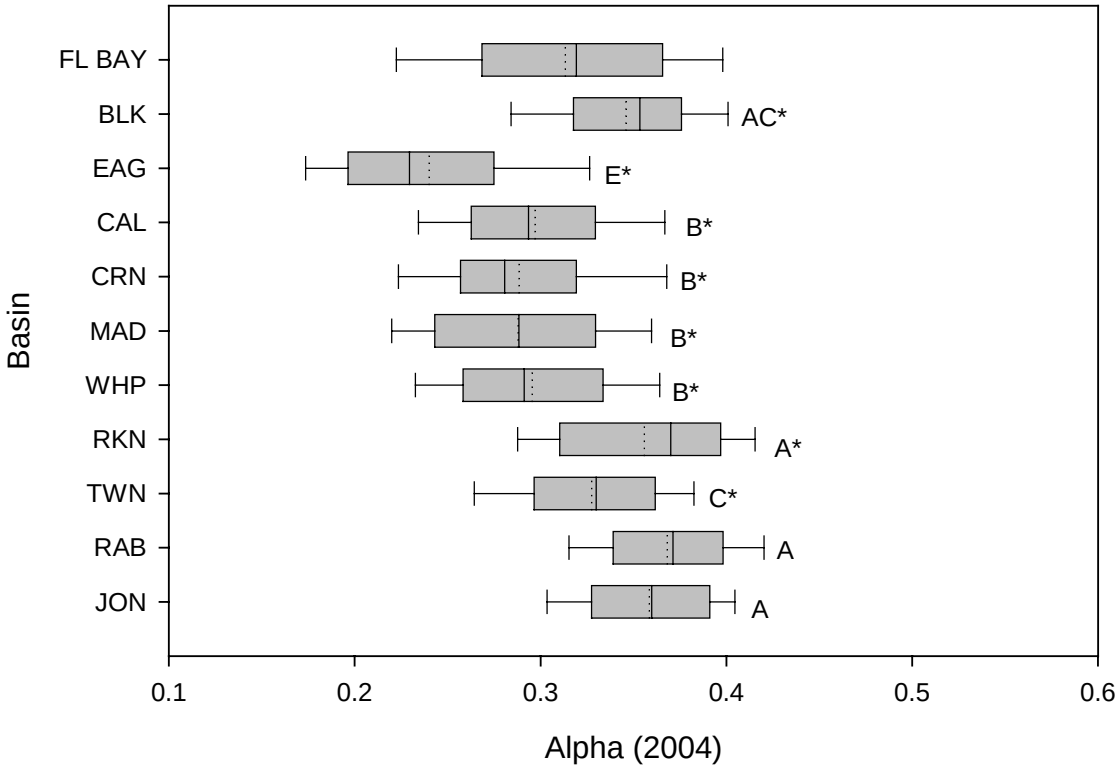
Alpha (2002)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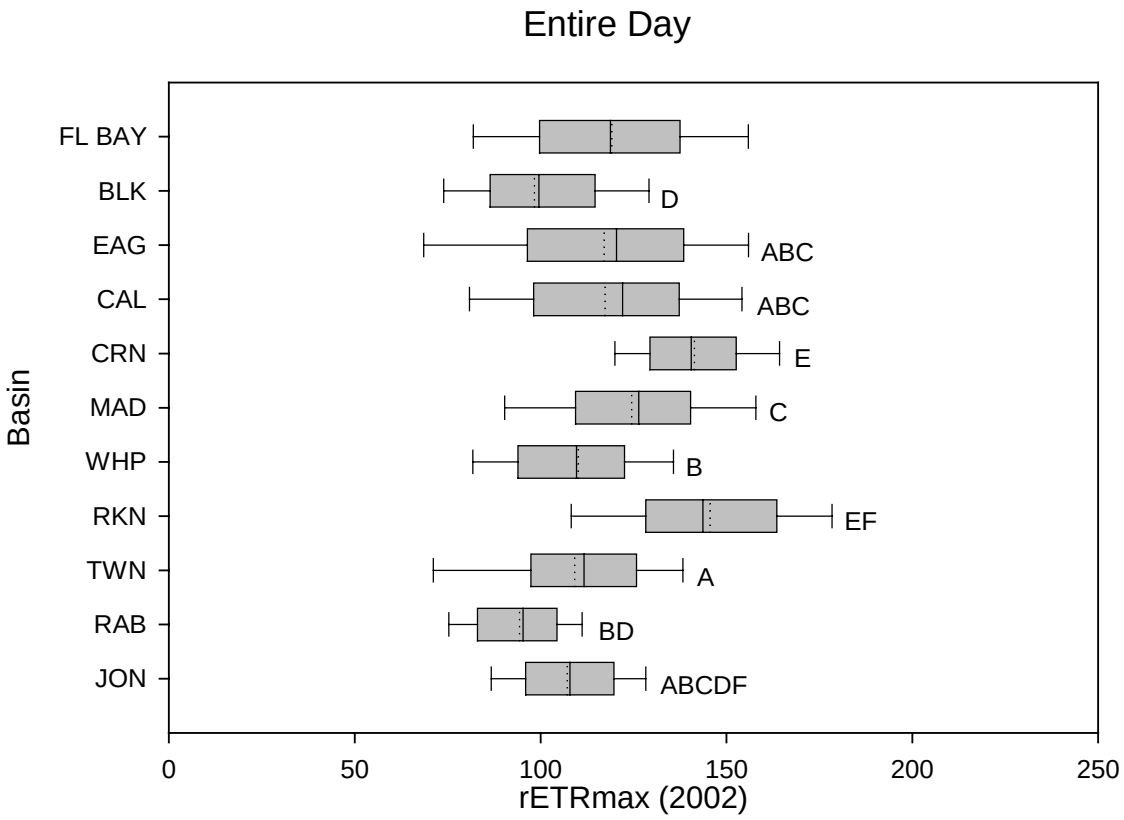
basin variability, but there was a similar range in both years (Figure 12). Mean daily rETRmax displayed as much or more variation among basins than any separate time period alone in both 2002 and 2004 (compare Figures 12 and 9).

The basins were arranged graphically (Figures 8-12) relative to their spatial position with in the bay (east to west) in order to determine if photosynthetic parameters reflected the gradient of environmental conditions within the bay. Excluding Blackwater Sound, there is a general trend of increasing effective yield and alpha from east to west within the bay, which was most pronounced in 2004, during mid-day, afternoon and all day comparisons.

Though Blackwater Sound is located farthest east in the bay the photosynthetic characteristics of *T. testudinum* in the Sound more closely resemble the basins located in the north central. rETRmax exhibited an opposite trend of decreasing capacity from east to west within the bay, though this trend was less apparent.

DISCUSSION

Photosynthesis can respond to stochastic environmental fluctuations using an array of processes that affect light-harvesting efficiency and photosynthetic capacity. Regulation can be accomplished by variations of the relative abundance of the constituents of the photosystems or on a shorter timescale by varying the efficiency of their activities (MacIntyre et al. 2000). Plants have a range of adaptations, which allow them to change photosynthetic activity and capacity in response to prevailing light conditions. Structural and functional adaptations to long-term light conditions, including changes in pigment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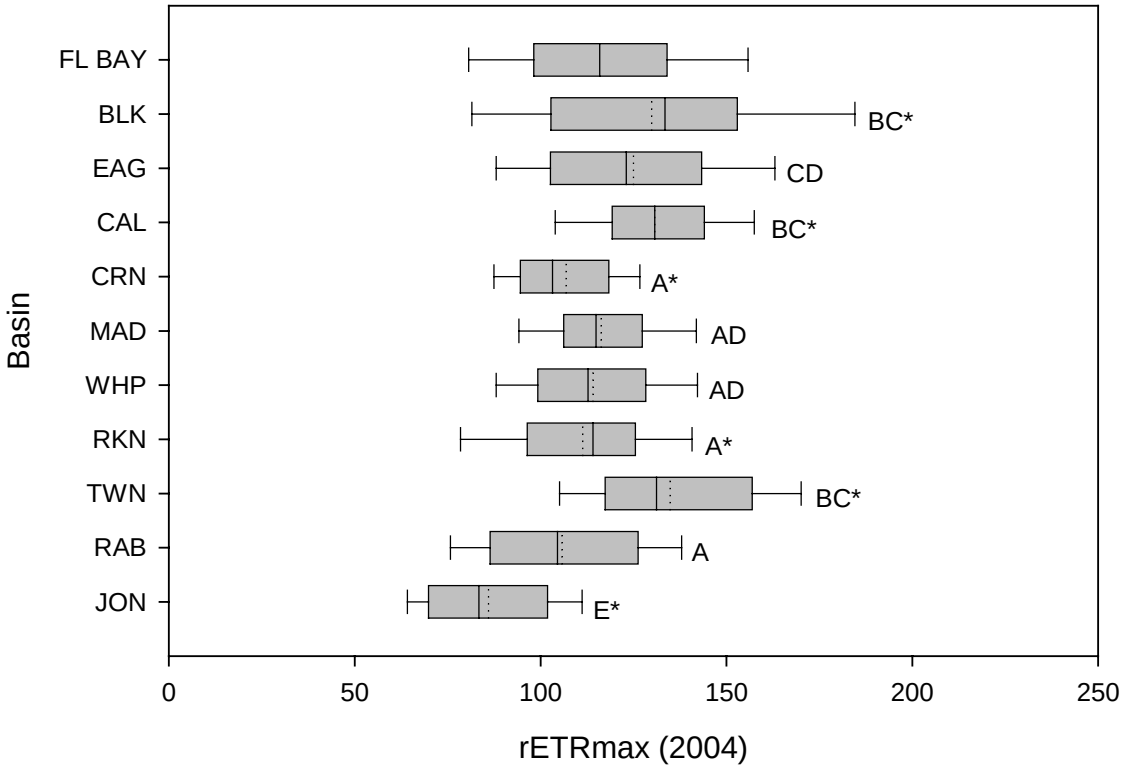
rETRmax (2002)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and protein (Rubisco) concentrations, determine the photoacclimation of the plant (sun-shade). There is also a complex group of rapid responses, which either decrease the absorption of light energy or provide alternative energy sinks when photosynthetic capacity is exceeded (MacIntyre et al. 2000). These protective processes (down-regulations) appear to limit damage to the photosystems but result in lower quantum yields (Gorbunov et al. 2001). Down regulations of photosynthesis allow plants to tolerate and utilize their rapidly changing light environment, while sustaining their photosynthetic system (White and Critchley 1999; Gorbunov et al. 2001). When protective responses are exceeded, proteins in the reaction centers can be damaged by excess excitation energy and re-synthesis of these proteins is required (MacIntyre et al. 2000). The levels of regulation of photosynthetic processes can be distinguished by timescales needed for them to take place and recover. Yet the occurrence and magnitude of environmental changes dictate the relative importance of the regulatory mechanisms. Because environmental factors such as light and temperature vary independently from photosynthetic changes, and on timescales that coincide with these changes, the results can be complex and unpredictable (MacIntyre et al. 2000).

Our results demonstrate that effective yield and rapid light curve derived photosynthetic parameters (alpha and rETRmax) vary throughout the day, yet the statistical significance of the variations were inconsistent among basins and years. Effective yield and alpha provide a measure of the efficiency of photon absorption and both exhibited similar diurnal patterns in this study. The highest values of both parameters were observed in the morning with a marked decrease during mid-day. In some basins there was a partial recovery in the afternoon, while in others there was a

continued decrease as the day progressed. In the basins that had a partial recovery in the afternoon we believe that this rapid recovery time indicates downregulation and photoprotection had occurred, which prevented damage during high mid-day irradiances. But the continued decrease in effective yield and alpha observed in other basins suggests photodamage. In these basins, there may not have been enough time following the highest mid-day irradiances to reestablish the pool of functional reaction centers by the time our sample period ended. Beer et al. (1998) measured effective quantum yields of shallow and deep growing coral colonies, over a diurnal cycle. They found that calculated ETR values for shallow high-light colonies were always lower in the afternoon than in the morning, at equal irradiances. Also, shallow colonies consistently had lower quantum yields than deeper colonies. These results, along with our observations, indicate there is a trade off in photosynthetic efficiency for photoprotection. Both effective yield (-0.63 $p<0.0001$ df=1054) and alpha (-0.57 $p<0.0001$ df=1054) were negatively correlated with the ambient irradiance at the seagrass canopy (PAR_{canopy}). There was a marked increase in irradiance reaching the seagrass canopy in 2004 in the majority of the basins. Coinciding with the increases in irradiance was an increase in the number of basins that exhibited a significant variation in PAR_{canopy} throughout the day. This trend illustrates that as the magnitude of changes in the driving environmental parameter increased there was a corresponding increase in the magnitude of the regulatory mechanisms.

The capacity of photosynthesis (rETRmax) also exhibited a consistent diurnal pattern and the significance of the changes varied among basins and years. Photosynthetic capacity was lowest in the morning and steadily increased as the day

progressed, resulting in the highest capacity in the afternoon. There were a few exceptions to this general trend though they were not significant. In CRN in 2002 and EAG in 2004 there was a decrease in rETRmax from morning to mid-day, but both basins exhibited an increase in afternoon. The largest deviation from the general trend was in CRN in 2004, which showed the exact opposite trend as the majority of other basins. We believe this was an artifact due to the fact that we experienced battery problems with the Diving-PAM and had to split up the sample period into two days. rETRmax was most closely correlated (0.49 $p<0.0001$ $df=1054$) with temperature, and both increased in a very similar way throughout the day. The rate of photosynthesis at light saturation is assumed to be dependant on the activity of Rubisco, which can be controlled by variations in the enzyme's concentration or in the short term by its activation state (MacIntyre et al. 2000). The observed pattern may reflect increased synthesis of Rubisco, which could result in an accumulation of enzyme as the day progresses and a subsequent increase in carbon fixation capacity. Most enzyme-catalyzed reactions show an exponential increase in rate as temperature increases (Taiz and Zeiger 1998), which could also explain the consistent morning to afternoon increases we observed. The general trend in photosynthesis of *T. testudinum* was increased capacity with decreasing efficiency as the day proceded. This resulted in a negative correlation (-0.21 $p<0.0001$ $df=1054$) between alpha and rETRmax and it suggests that the excess excitation energy created by high irradiances may be preferentially hindering the efficiency of the light reaction.

Though measurements taken with PAM fluorometry have a significant amount of diurnal variability, informative physiological patterns do emerge. Excluding Blackwater

Sound, which is the most eastern basin within the bay, yet has been found to be more similar to the north central basins, there is a general east to west trend of increasing photosynthetic efficiency, which correlates with previous research on morphometric characteristics of *T. testudinum* within Florida Bay (Hackney and Durako 2004; Hackney and Durako 2005). They found that standing crop, the ratio of aboveground-to-belowground biomass, leaf area index, leaf number and size increase from east to west within the bay, which they attributed to landscape-scale differences in environmental parameters. In conjunction with increasing photosynthetic efficiency, a general trend of decreasing capacity was observed from east to west, within the bay. This gradient could be an indication of a photoadaptive response to the general increase in depth along this gradient and may reflect a transition from sun- to shade-adapted plants. The ability to distinguish significant differences among basins and to detect landscape-scale trends in photosynthetic characteristics within the bay that are consistent with structural trends indicates that PAM fluorometry may be useful as a monitoring tool and can potentially detect signs of physiological stress before morphological changes take place. However, the results here clearly indicate that time of day effects must be factored into the experimental design and interpretation of PAM fluorescence data.

Conclusion

Although PAM fluorometers can readily provide in-depth physiological information, interpreting the results is not always straightforward. Rapid light curves were found to be as sensitive as effective yields to diurnal fluctuations, even though they take measurements over a range of irradiances. Accurate physiological information can be masked by diurnal variations caused by the photosystems' dynamic response to

changing environmental conditions. In ecosystems where the magnitude of changes are large and occur on much faster time scales than the ecosystem changes being measured, the resulting fluctuations may obscure the true physiological signal. Therefore, when using PAM fluorometers over large spatial and temporal scales diurnal variability must be considered.

Rigorous statistical testing was unable to discern which time of day was best suited for assessing the photophysiological status of *T. testudinum* in Florida Bay. It may be preferable to restrict measurements to the morning before irradiance rises to the mid-day maximum, but after the extended dark period of night, which allows time for reactions centers to all be available and for repair of any photodamage. On the other hand, in order to detect the effects of stress it may be wise to do assessments after the high light stress of mid-day. When taking virtually simultaneous replicate measurements of effective yield over a diel cycle Runcie and Durako (2004) found that measurements taken during mid-day exhibited the highest variability (~25% of the mean), which in turn decreased their ability to detect changes in efficiency. In addition, in certain seagrass species, calculated ETRs based on fluorescence measurements have a linear relationship with O_2 evolution, but for other species they only correlate at lower irradiances, with increasing discrepancies at higher irradiances (Beer et al. 1998; Beer and Björk 2000). Considering this, using PAM fluorometry at a time and irradiance when it most closely correlates with oxygen evolution may provide a clearer physiological signal. However, when doing landscape-scale assessments one must factor in both temporal and spatial variation. In order to evaluate the status of an ecosystem, it may be better to comprehensively cover the entire spatial scale of

sampling. Using this point of view, evaluating mean daily characteristics and consequently using data from the entire spatial sample might provide more representative scale-appropriate information.

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CHAPTER 2

EXPLORING DIEL LIGHT CURVES IN ORDER TO INCORPORATE TEMPORAL AND SPATAIL VARIATION

INTRODUCTION

Pulse-Amplitude Modulated (PAM) fluorometry provides almost instantaneous *in situ* measurements of a variety of photosynthetic characteristics, under ambient light conditions. With the development of an underwater fluorometer, Diving-PAM (Walz, Germany), it is now possible to study photosynthesis of aquatic organisms, such as seagrasses, without the use of gas-exchange enclosures (Beer et al. 1998). The measurement of chlorophyll fluorescence, emitted from PSII, provides insight into changes in photochemistry, and permits the study of effects of varying environmental conditions on photosynthetic reactions (White and Critchley 1999). Also, PAM fluorometry is an attractive tool because it is rapid, non-destructive, and can provide in-depth, quantitative physiological information about an organism.

When incorporating PAM fluorometry into the landscape scale assessment of seagrass in Florida Bay, during the Fish Habitat Assessment Program (FHAP), diurnal variation became evident (see Durako et al. 2002 for more information on FHAP). The first indication of temporal variability of the physiological signal was the significant negative slopes in regressions of effective and maximum quantum yield against ambient irradiance or time of day (Durako and Kunzelman 2002). Further investigation (Chapter 1) revealed that effective quantum yield and rapid light curve derived parameters (alpha and rETRmax) also exhibited diurnal variability, but the significance of the variations were inconsistent among basins and years. Florida Bay is a subtropical lagoonal estuary and resident organisms are exposed to a large range of physical parameters

(irradiance and temperature) throughout the day. Photosynthesis can rapidly respond to changes in the environment (Macyntyre et al. 2002). The sensitivity of PAM fluorometry in detecting rapid changes in photochemistry may thus result in an obscured physiological signal. This confounded the physiological assessment of the eco-indicator species *Thalassia testudinum* Banks ex König (Hydrocharitaceae) in Florida Bay (Chapter 1). Though some informative physiological patterns did emerge, rigorous statistical testing was unable to determine the most suitable time to take photosynthetic measurements. It was concluded that when doing ecosystem-level assessment covering the entire spatial scale provides more representative information, though temporal variation is still a factor.

Prompted by discrepancies between PAM fluorescence and O_2 evolution techniques, Longstaff et al. (2002) investigated the accuracy of PAM fluorometry in assessing *in situ* photosynthetic rates. They took measurements with an automated O_2 exchange apparatus, performed rapid light curves and obtained point measurements of effective yield over a diel period. By constructing traditional photosynthesis-irradiance (P-E) curves using O_2 measurements and fluorescence-based diel light curves using the Diel Yield and Diel Rapid Light Curve (RLC) method, they were able to generate comparable curves that spanned the entire temporal scale of sampling. They found that under certain conditions and with some limitations (mainly at high irradiance levels) PAM fluorescence could accurately assess photosynthetic rates of the simple laminate algae, *Ulva lactuca* Linnaeus.

The use of diel light curves was investigated in the current study in order to incorporate both the temporal and complete spatial scale. The objective was to discern

the usefulness of diel light curves for overcoming methodological constraints of landscape-scale assessment and also to assess their effectiveness in detecting the physiological condition of the seagrass *T. testudinum*.

Null Hypotheses:

$H_0$3: Diel light curves calculated by the diel yield method and diel rapid light curve method will not significantly differ from each other among basins and years

$H_0$4: Diel light curves calculated by the diel yield method will not significantly differ among basins and years

$H_0$5: Diel light curves calculated by the diel rapid light curve method will not significantly differ among basins and years

METHODS

Study site

This study was conducted in Florida Bay (ca. 25°05'N, 81°45'W), which is a shallow lagoonal estuary at the southern tip of Florida, USA. The bay is characterized by shallow basins (ca. <1m) divided by carbonate mud banks and mangrove islands (Fourqurean and Robblee 1999). During FHAP, 10 basins were sampled that lie within the borders of the Everglades National Park (ENP) (Table 1). The basins were chosen to represent the range of conditions within the bay. Each basin was divided into 27-33 tessellated hexagonal subunits, and one station was randomly chosen within each subunit. This resulted in 275-330 stations that were randomly sampled throughout the bay (Figure 1). As a result of the large sample area, stations must be sampled systematically in order to minimize station-to-station travel time, yet it still took an entire day (8am to 5pm) to sample one basin.

Table 1. List of basins sampled in Florida Bay and their abbreviations.

Abbreviation	Basin
BLK	Blackwater Sound
CAL	Calusa Key
CRN	Crane Key
EAG	Eagle Key
JON	Johnson Key
MAD	Madeira Bay
RAB	Rabbit Key
RKN	Rankin Lake
TWN	Twin Key
WHP	Whipray Bay

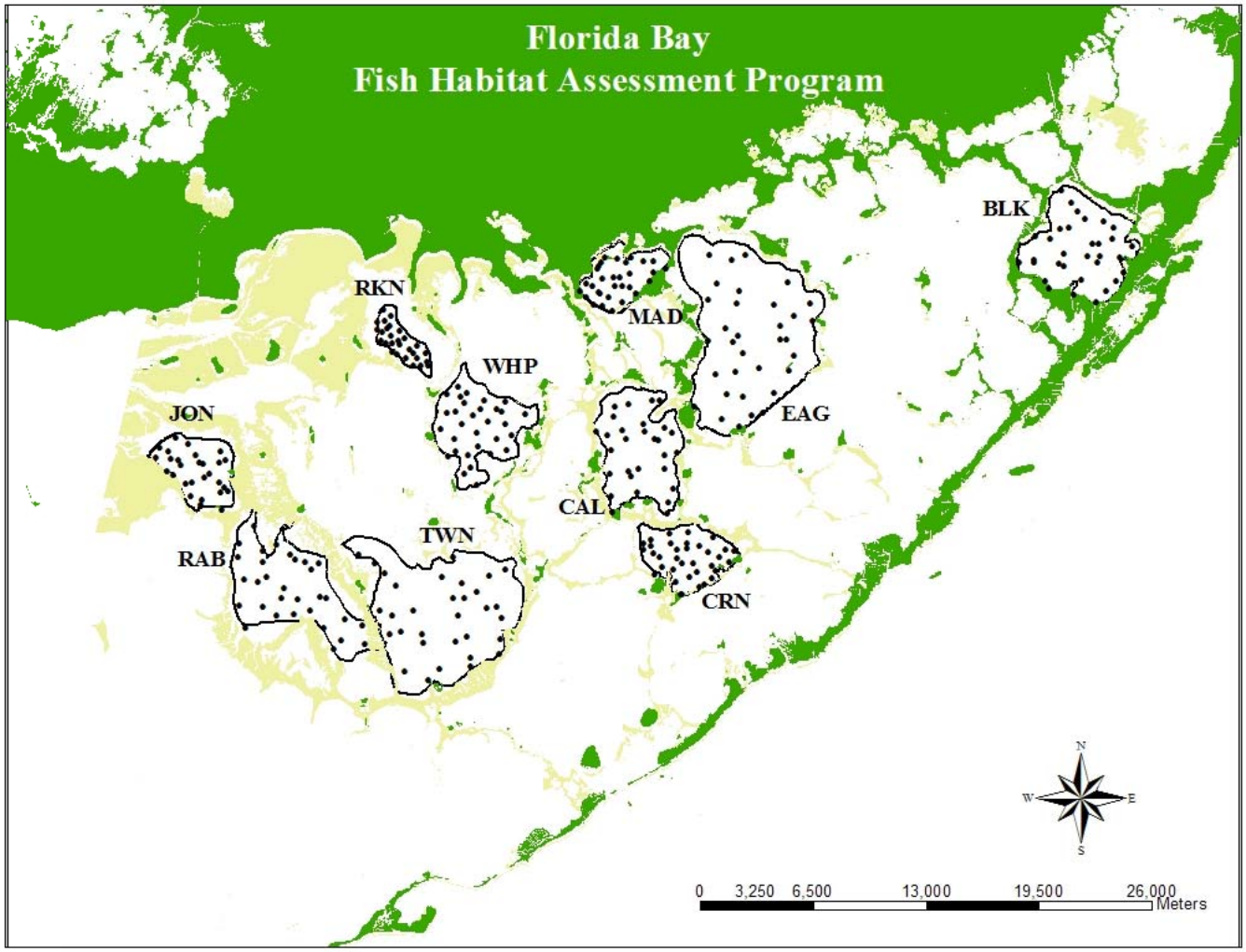


Figure 1. Map of basins and sample stations within Florida Bay

Sampling technique

Photosynthetic characteristics were measured using an underwater fluorometer, Diving PAM (Walz, Germany), in 2002 and 2004, during the spring FHAP sampling. Rapid light curves (RLC) were performed on four haphazardly chosen short shoots of *T. testudinum* at each station. The short shoots that were chosen were representative of the shoots observed at each station. The middle of the rank 2 blade of each *T. testudinum* short shoot was gently scraped to remove epiphytes before attaching the dark leaf clip (DIVING-LC). The leaf clip held the Diving PAM fiber optic 5mm from the surface of the blade in 2002. This distance was reduced to 2mm in 2004 in order to allow for a reduction in instrument gain and a higher signal to noise ratio. Each rapid light curve exposed the leaf to eight incremental steps of irradiance ranging from 0 to 2060 µmol photons $m^{-2}s^{-1}$ in 2002, and 0 to 1735 µmol photons $m^{-2}s^{-1}$ in 2004. The reduction in irradiance levels in 2004 was due to slight damage to the fiber optic causing a decrease in light transmission at the same instrument settings. An effective yield measurement (F_m'-F_o'/F_m') was taken at the beginning of each curve, before light was applied, and at the end of each 5s irradiance step, resulting in nine yield measurements for each rapid light curve performed. Each yield measurement was used to calculate the relative electron transport rate (rETR) through photosystem II using the following equation: rETR=Yield x PAR x AF x 0.5, where PAR is the light generated by the internal halogen lamp of the Diving PAM, AF is the fraction of light absorbed by the leaf, and 0.5 assuming that the photons absorbed are equally partitioned between PSII and PSI (Genty et al. 1989). Note that this is only a relative rate of electron transport since the default AF value of 0.84, set for terrestrial plants, was used in this study.

To assess the diurnal changes in photosynthesis in response to changing ambient irradiances, two types of diel light curves were calculated, the Diel Rapid Light Curve (RLC) method and the Diel Yield method (Longstaff et al. 2002). For the Diel Rapid Light Curve method, RLCs were taken throughout the sample period (~0800-1700h) along with measurements of the ambient irradiance at the seagrass canopy, measured using a quantum PAR scalar sensor (LiCor LI-193S). rETRs were calculated for all nine yield measurements in the RLC using the internally generated irradiances. The rapid light curve produced using the internal actinic irradiance was used to interpolate the rETR at the measured ambient irradiance taken at the time of the RLC. The interpolated rETR's and the ambient irradiances were plotted in order to create a diel P-E curve for each sample basin and a nonlinear regression was used to quantify certain aspects of the curve (alpha, rETRmax, I_K). The initial slope of the curve (alpha) is a measure of the light harvesting efficiency of photosynthesis. The asymptote of the curve, the maximum rate of photosynthesis (rETRmax), is a measure of the ability of the photosystems to utilize the absorbed light energy (Marshall et al. 2000). The minimum saturating irradiance (I_k) can be calculated by the following equation: I_k =alpha/ $ETR_{max,}$ and is an indicator of the photoacclimation state of the plant (Ralph and Gademann 2005). For the Diel Yield method, effective quantum yields (the first yield measurement of the RLC taken before light is applied) and ambient irradiances were measured throughout the sample period (~0800- 1700 h). Then rETRs were calculated from the first effective yield measurement and the ambient irradiance at the time of the measurement. Nonlinear regressions of the subsequent rETRs and the ambient

irradiance were also calculated. All nonlinear regressions where done in Sigmaplot 9.0 using a user-defined double exponential decay function (Platt et al. 1980).

Analysis

Diel light curves generated by the Diel Yield and Diel RLC methods were compared within each basin and between years (2002, 2004). In order to compare the two methods and assess their ability to predict the relationship between calculated rETR and ambient irradiance (PAR), nonlinear regressions were calculated. Linear regressions of the residuals of each non-linear regression and the independent variable (PAR) were also calculated to determine if either model exhibited irradiance-based bias. In order to determine if the two methods were significantly different from each other a linear regression of the difference between the data sets was plotted against the independent variable (PAR).

RESULTS

Fluorescence-based photosynthetic irradiance (P-E) curves constructed for the ten basins sampled in FHAP during 2002 and 2004 enabled the comparison of the Diel Yield and Diel RLC methods. A linear regression of the difference between the calculated rETR's of the two methods (Diel Yield-Diel RLC) and the independent variable (PAR) determined that the curves generated from the two methods were statistically different from one another (r^2=0.785, $p<0.001$; Figure 2) and that the difference increased with irradiance. Linear regressions of the residuals of each individual P-E curve plotted against the independent variable (ambient PAR) indicated

no irradiance-based bias in the model fit for either method (data not shown). All non-linear regressions, which generated the P-E curves for the two methods, were significant except one calculated for the Diel RLC method for EAG in 2004 (Table 2). Regressions of curves constructed with the Diel Yield method generally had a much higher r^2 than the Diel RLC method (Table 2).

For all basins and between both years, the two methods predicted different relationships between electron transport rates (rETR) and irradiance (Table 2). Trends emerged that rendered information about the usefulness and drawbacks of the two methods. For the most part, the P-E curves generated for both of the methods exhibited consistently different patterns in all of the basins (Figures 3-12). With the Diel Yield method, rETR increased as irradiance increased, which resulted in very high levels of electron transport and rETRmax's that ranged from 137-1349 (μmol electrons $m^{-2}s^{-1}$). In contrast, the Diel RLC method ETR versus PAR curves generally reached an asymptote at higher irradiances and resulted in much lower predictions of rETRmax, which ranged from 66-140 (μmol electrons $m^{-2}s^{-1}$). The initial slopes of the curves (alpha) calculated from both methods were comparable within most basins, though the Diel Yield method usually had slightly higher alphas (Table 2). Calculated values of I_K were, for the most part, substantially higher (ranging from 297-4813 μmoles quanta $m^{-2}s^{-1}$, with the exception of CRN in 2004) with the Diel Yield method than the Diel RLC, which ranged from 28-599 μmoles quanta $m^{-2}s^{-1}$ (Table 2).

All of the Diel Yield method curves increased as irradiance increased but the shape of the curves varied among basins and years. There were three basic shapes that resulted

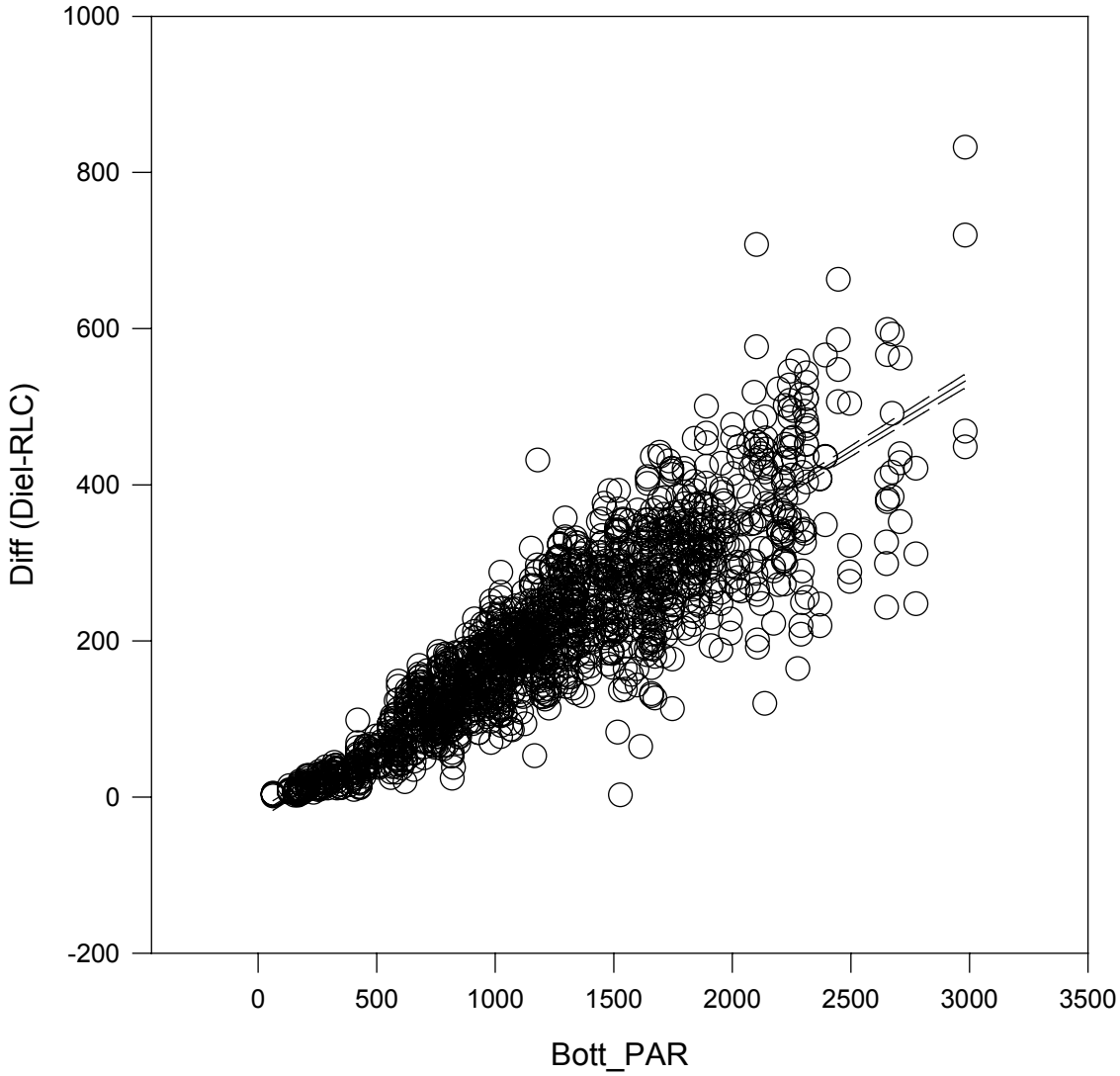
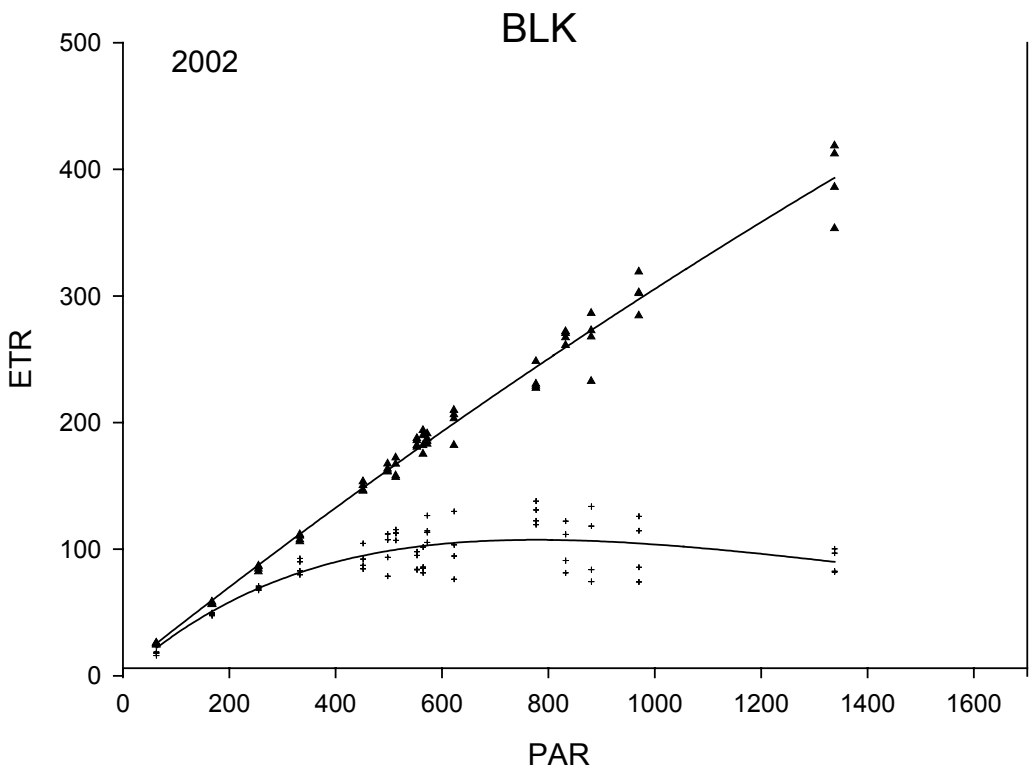


Figure 2. Linear regression of the difference between the calculated rETR's of two methods (Diel Yield-Diel RLC; includes all basins and both years) vs. the ambient irradiance measured at the seagrass canopy (PAR µmol photon $m^{-2}s^{-1}$).

Year	Basin	Diel RLC method						Diel Yield method					
		P-E Curve		Residual				P-E Curve		Residual			
		r^2	P value	P value	alpha	rETRmax	IK	r^2	P value	P value	alpha	rETRmax	Ik
2002	**BLK**	0.732	<0.001	0.848	0.38	107.33	285.55	0.986	<0.001	0.821	0.33	1193.53	3575.82
	CAL	0.738	<0.001	0.891	0.35	121.68	351.73	0.969	<0.001	0.931	0.35	223.47	636.51
	CRN	0.579	<0.001	0.981	0.33	140.23	424.94	0.908	<0.001	0.522	0.33	667.52	2047.21
	EAG	0.683	<0.001	0.739	0.34	128.87	382.13	0.949	<0.001	0.772	0.36	497.14	1393.08
	JON	0.246	0.002	0.931	0.21	107.50	514.45	0.957	<0.001	0.994	0.42	152.14	358.80
	MAD	0.234	0.002	0.981	0.21	119.47	559.69	0.844	<0.001	0.964	0.38	318.40	847.18
	RAB	0.403	0.05	0.912	0.33	98.86	295.86	0.992	<0.001	0.822	0.46	137.38	297.34
	RKN	0.12	0.03	0.991	0.30	132.47	447.81	0.911	<0.001	0.181	0.29		
	TWN	0.763	<0.001	0.889	0.31	121.48	392.77	0.962	<0.001	0.494	0.36	557.86	1541.72
	WHP	0.745	<0.001	0.858	0.36	116.69	322.29	0.933	<0.001	0.843	0.37	417.62	1143.51
2004	**BLK**	0.421	<0.001	0.858	0.37	121.83	332.60	0.937	<0.001	0.737	0.39	208.76	538.34
	CAL	0.038	<0.001	0.999	3.91	112.91	28.90	0.777	<0.001	0.997	0.53	200.86	377.58
	CRN	0.065	<0.001	0.861	0.26	108.46	422.87	0.681	<0.001	0.884	6.14	140.85	22.94
	EAG	0	1	0.05	1.34	113.24	84.29	0.227	<0.001	0.968	0.43	278.04	646.81
	JON	0.266	<0.001	0.986	0.37	84.92	227.71	0.948	<0.001	0.85	0.34	192.80	573.47
	MAD	0.076	0.04	0.993	0.22	79.65	365.99	0.471	<0.001	0.935	0.47	356.87	762.54
	RAB	0.05	0.002	0.916	0.24	108.00	448.92	0.899	<0.001	0.995	0.43	158.83	371.67
	RKN	0.192	<0.001	0.448	1.52	66.31	43.57	0.816	<0.001	0.894	0.40	450.11	1130.40
	TWN	0.199	<0.001	0.948	0.42	108.76	258.34	0.747	<0.001	0.807	0.35	599.01	1712.20
	WHP	0.067	0.016	0.993	0.32	109.72	343.38	0.846	<0.001	0.827	0.28	1340.90	4813.56

Table 2. Results from non-linear regressions of photosynthetic irradiance curves (P-E) generated with the Diel RLC and Diel Yield methods, including derived parameters (alpha, rETRmax, Ik) for the ten basins sampled within Florida Bay in 2002 and 2004 and linear regressions of each curve's residuals and independent variable (PAR µmol photons m-2s-1).

Figure 3. Diel Yield (▲) calculated rETR vs. irradiance (PAR µmol photon $m^{-2}s^{-1}$) and Diel RLC (+) interpolated rETR vs. irradiance (PAR) for BLK in 2002 and 2004.


BLK
2002
ETR
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0
100
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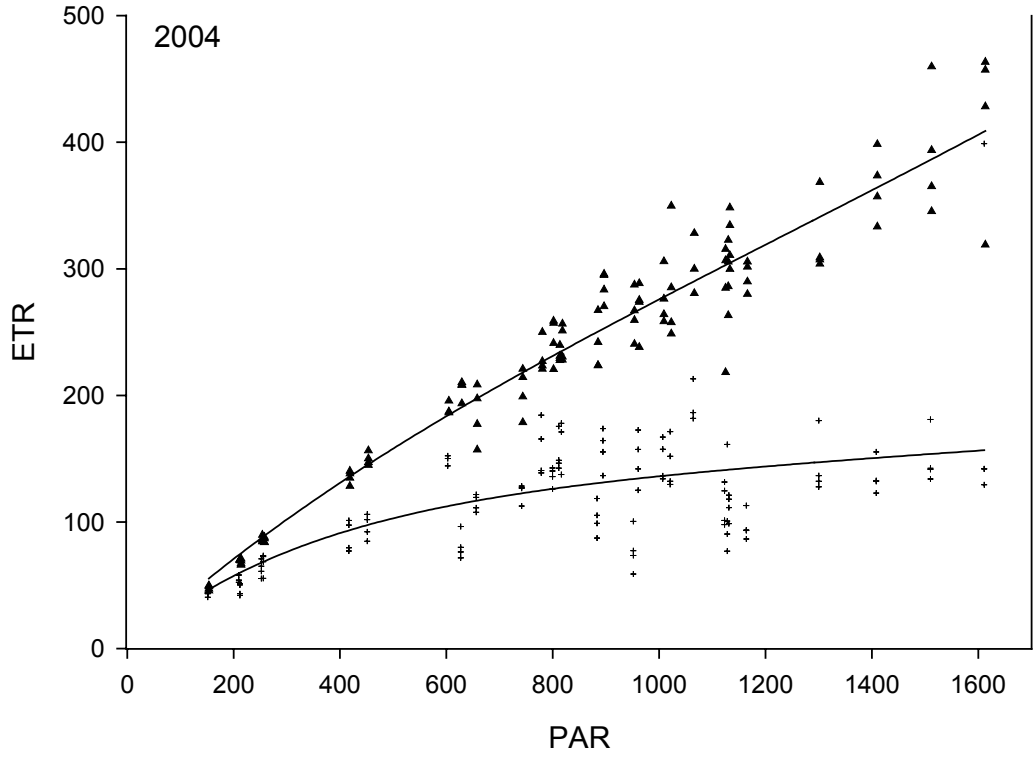
2004
ETR
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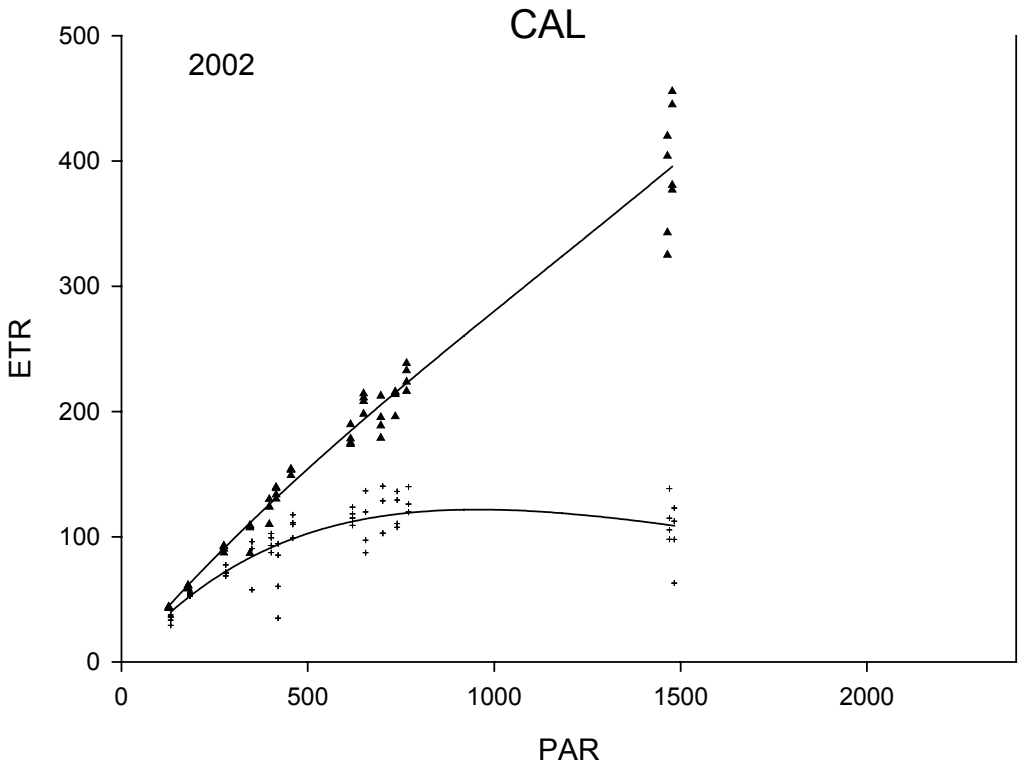
Figure 4. Diel Yield (▲) calculated rETR vs. irradiance (PAR µmol photon $m^{-2}s^{-1}$) and Diel RLC (+) interpolated rETR vs. irradiance (PAR) for CAL in 2002 and 2004


CAL
2002
ETR
PAR
0
100
200
300
400
500
0
500
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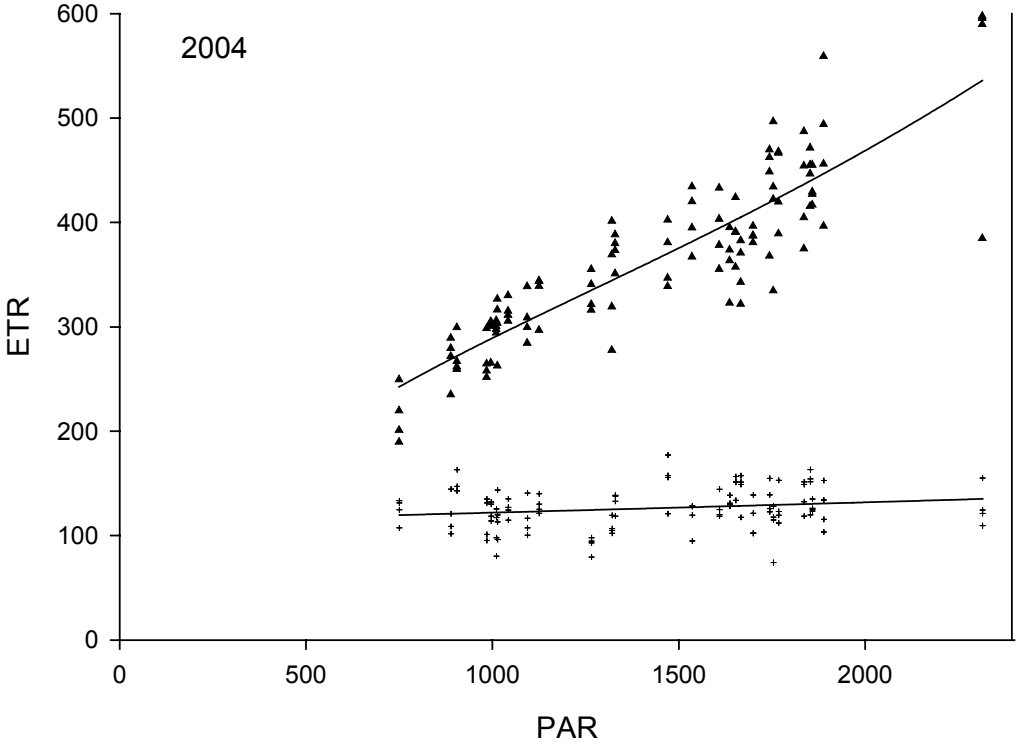
1500
2000


2004
ETR
PAR
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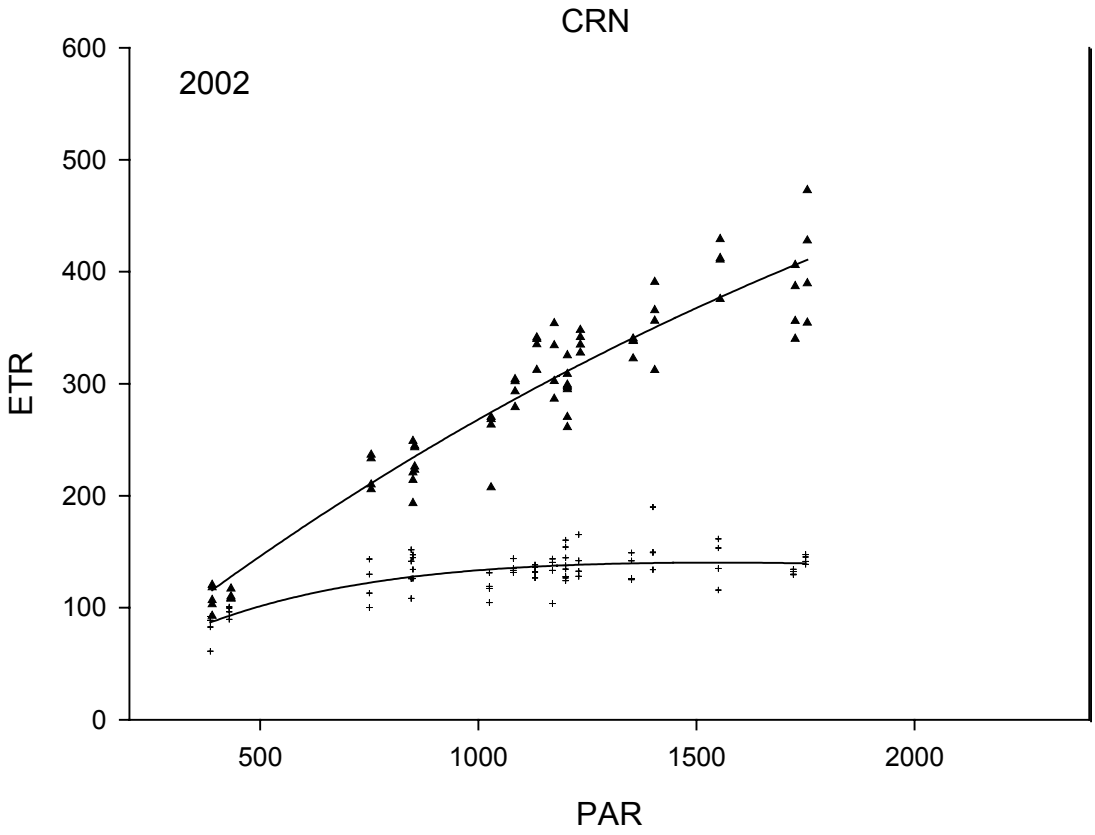
500
1000
1500
2000

Figure 5. Diel Yield (▲) calculated rETR vs. irradiance (PAR µmol photon $m^{-2}s^{-1}$) and Diel RLC (+) interpolated rETR vs. irradiance (PAR) for CRN in 2002 and 2004.


CRN
2002
600
500
400
300
200
100
0
ETR
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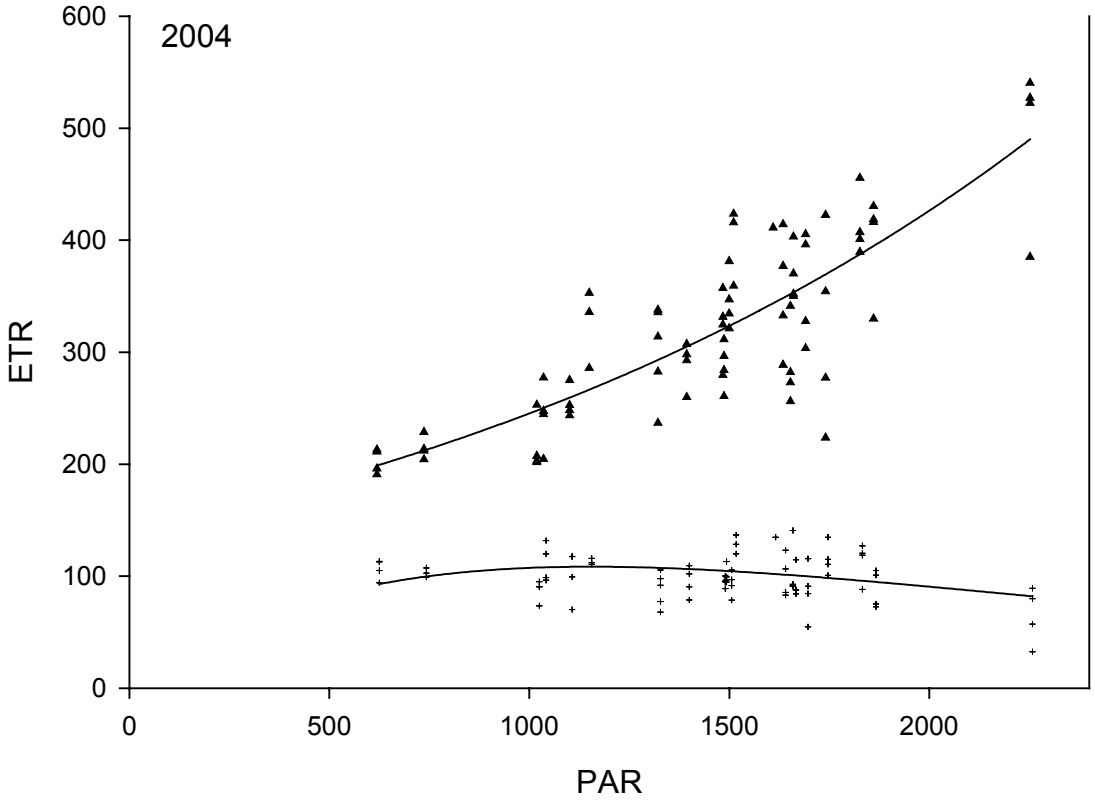
1000
1500
2000
PAR


2004
600
500
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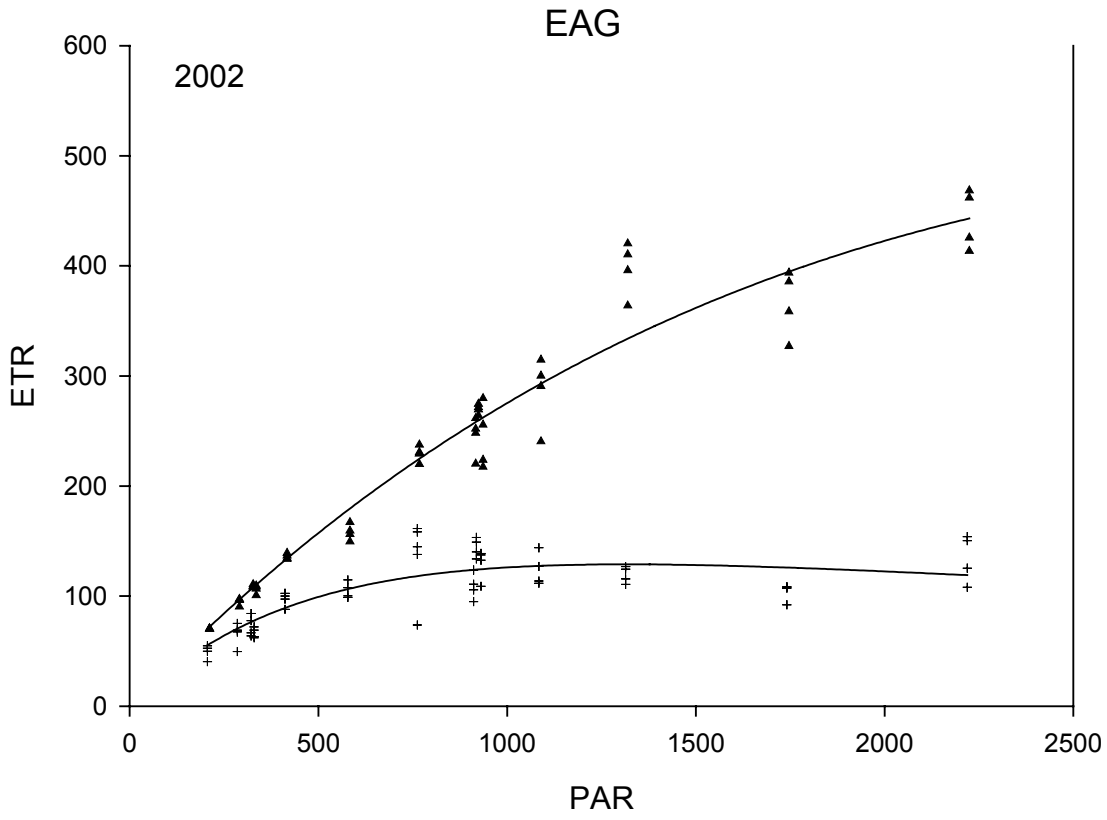
200
100
0
ETR
0
500
1000
1500
2000
PAR

Figure 6. Diel Yield (▲) calculated rETR vs. irradiance (PAR µmol photon $m^{-2}s^{-1}$) and Diel RLC (+) interpolated rETR vs. irradiance (PAR) for EAG in 2002 and 2004.


EAG
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ETR
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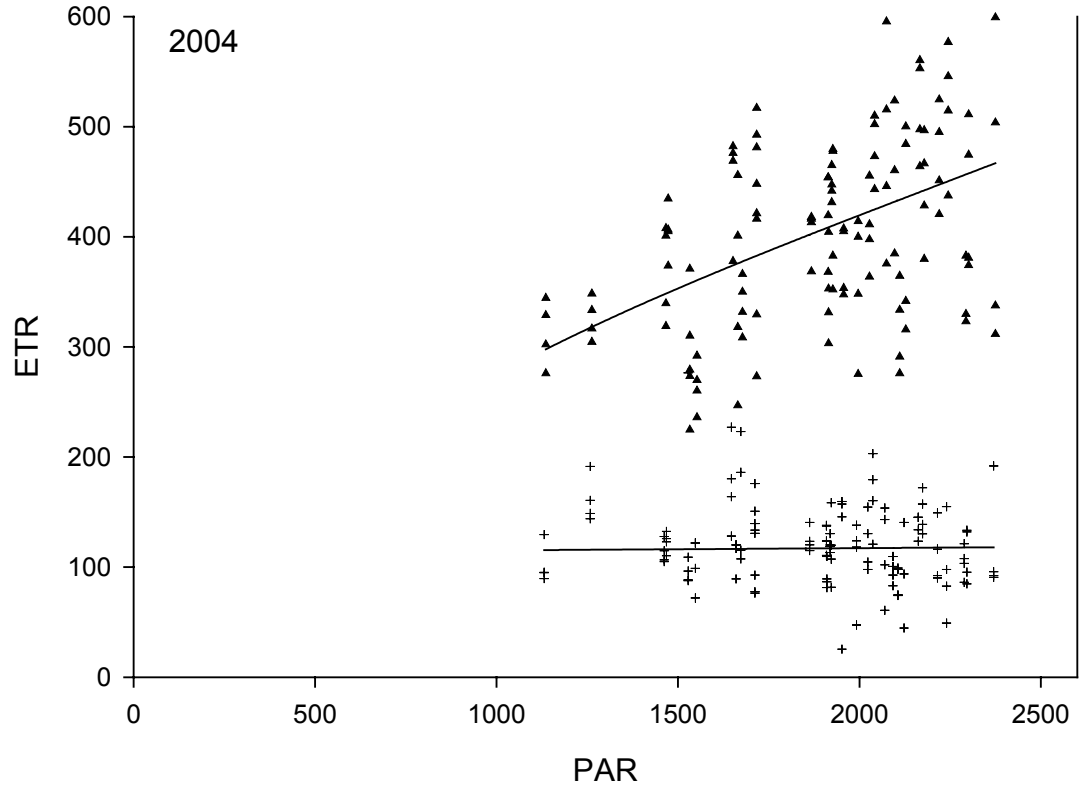
2500


2004
ETR
PAR
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100
200
300
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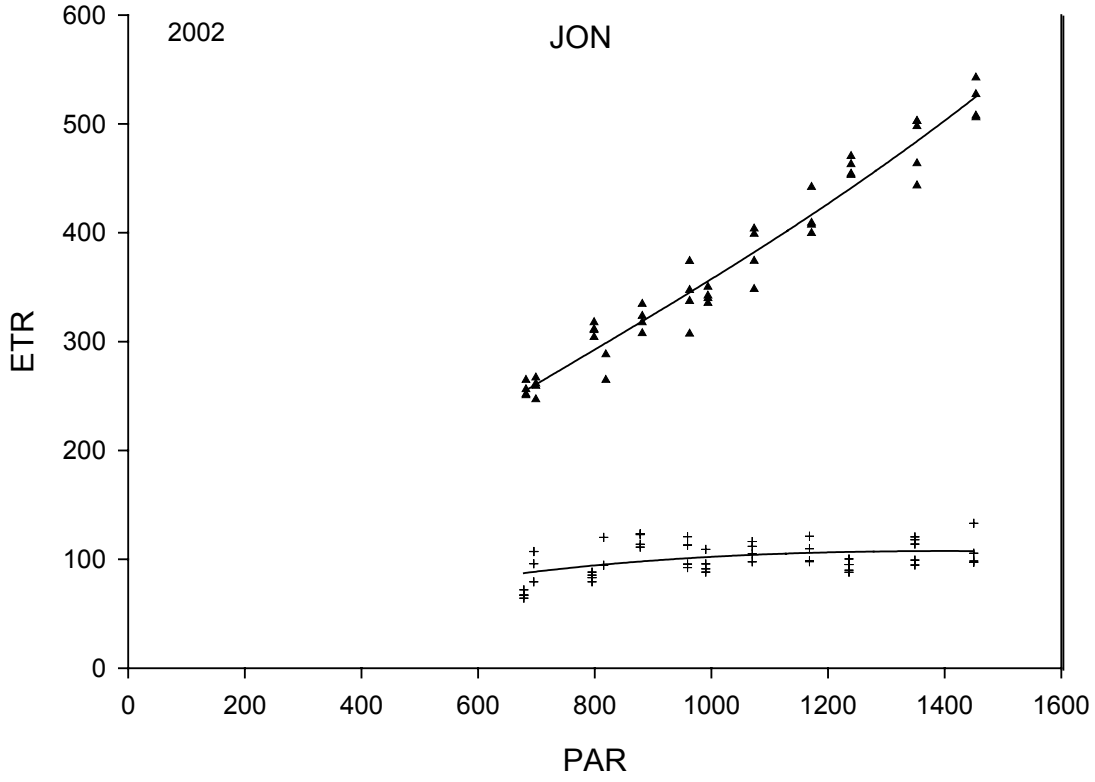
0
500
1000
1500
2000
2500

Figure 7. Diel Yield (▲) calculated rETR vs. irradiance (PAR µmol photon $m^{-2}s^{-1}$) and Diel RLC (+) interpolated rETR vs. irradiance (PAR) for JON in 2002 and 2004.


2002
JON
ETR
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800
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1200
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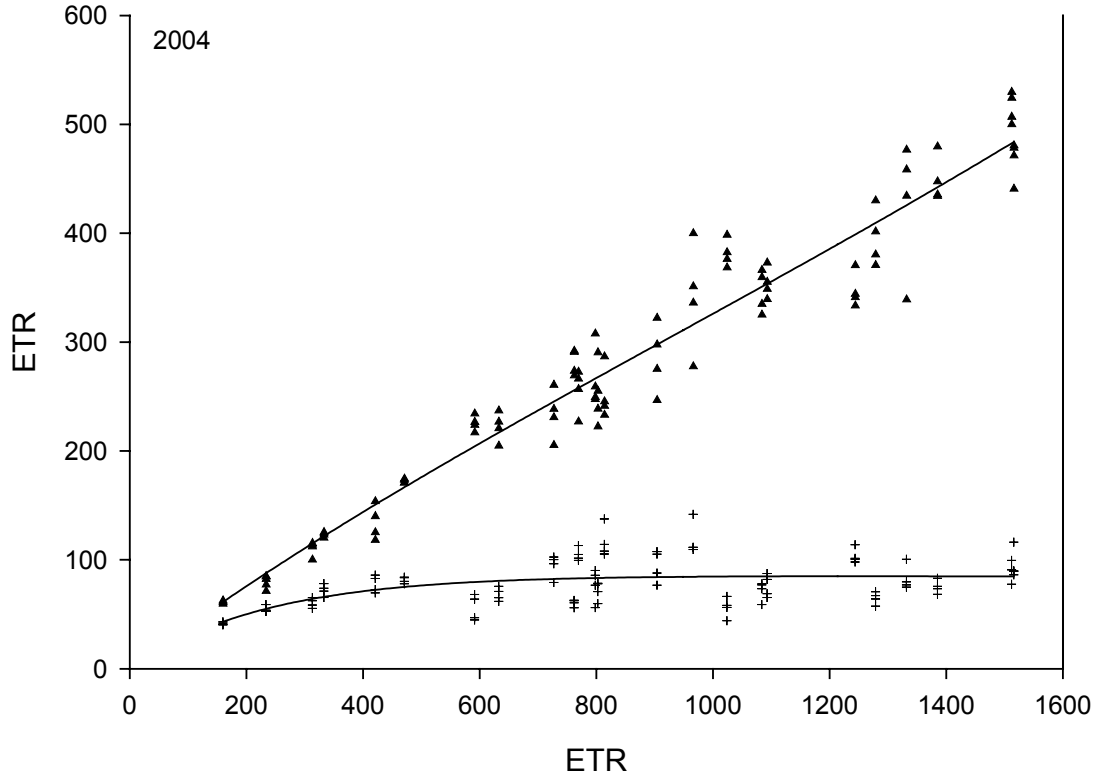
1600


2004
ETR
ETR
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600
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200
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800
1000
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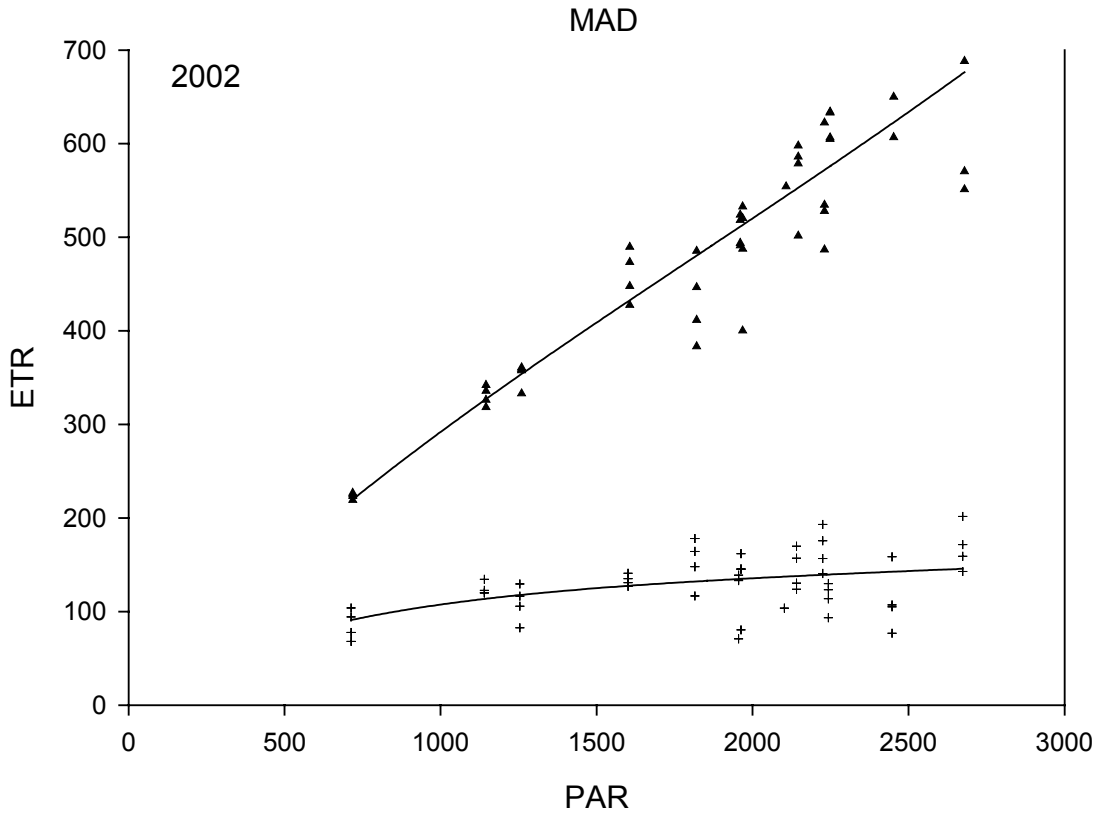
1400
1600

Figure 8. Diel Yield (▲) calculated rETR vs. irradiance (PAR µmol photon $m^{-2}s^{-1}$) and Diel RLC (+) interpolated rETR vs. irradiance (PAR) for MAD in 2002 and 2004.


MAD
2002
ETR
PAR
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100
200
300
400
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700
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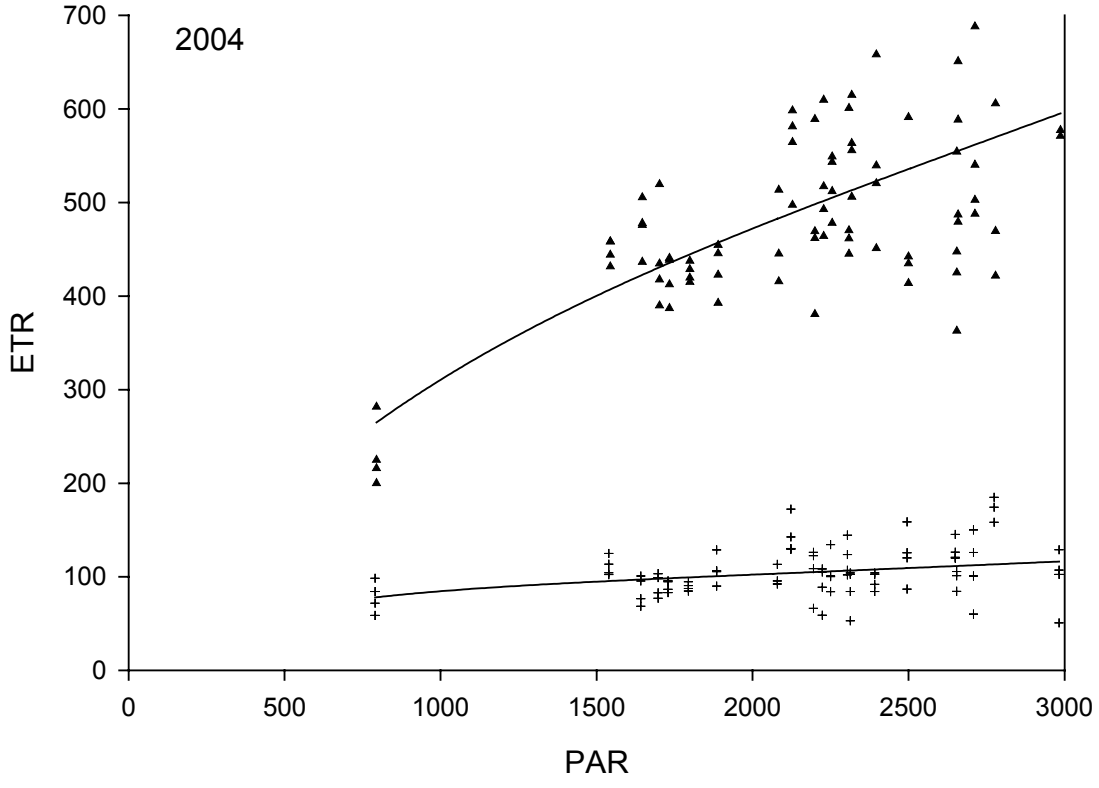
2000
2500
3000


2004
ETR
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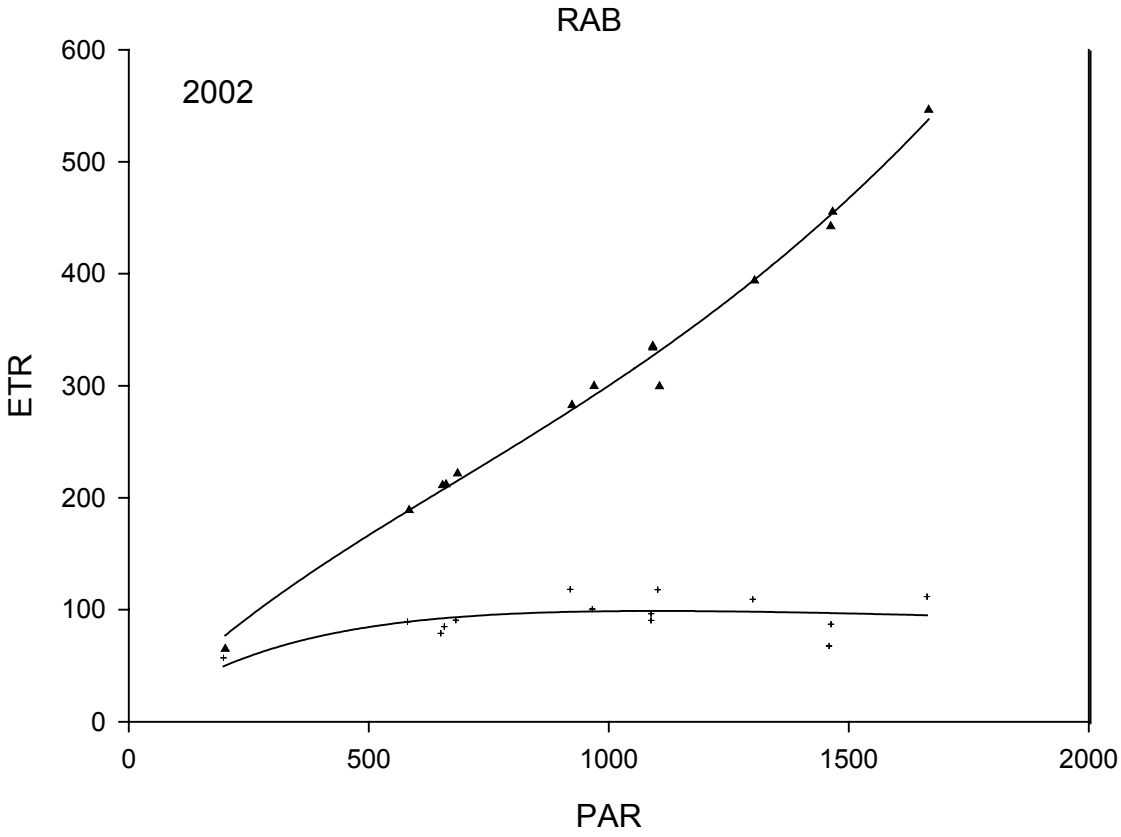
2000
2500
3000

Figure 9. Diel Yield (▲) calculated rETR vs. irradiance (PAR µmol photon $m^{-2}s^{-1}$) and Diel RLC (+) interpolated rETR vs. irradiance (PAR) for RAB in 2002 and 2004.


RAB
2002
ETR
PAR
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100
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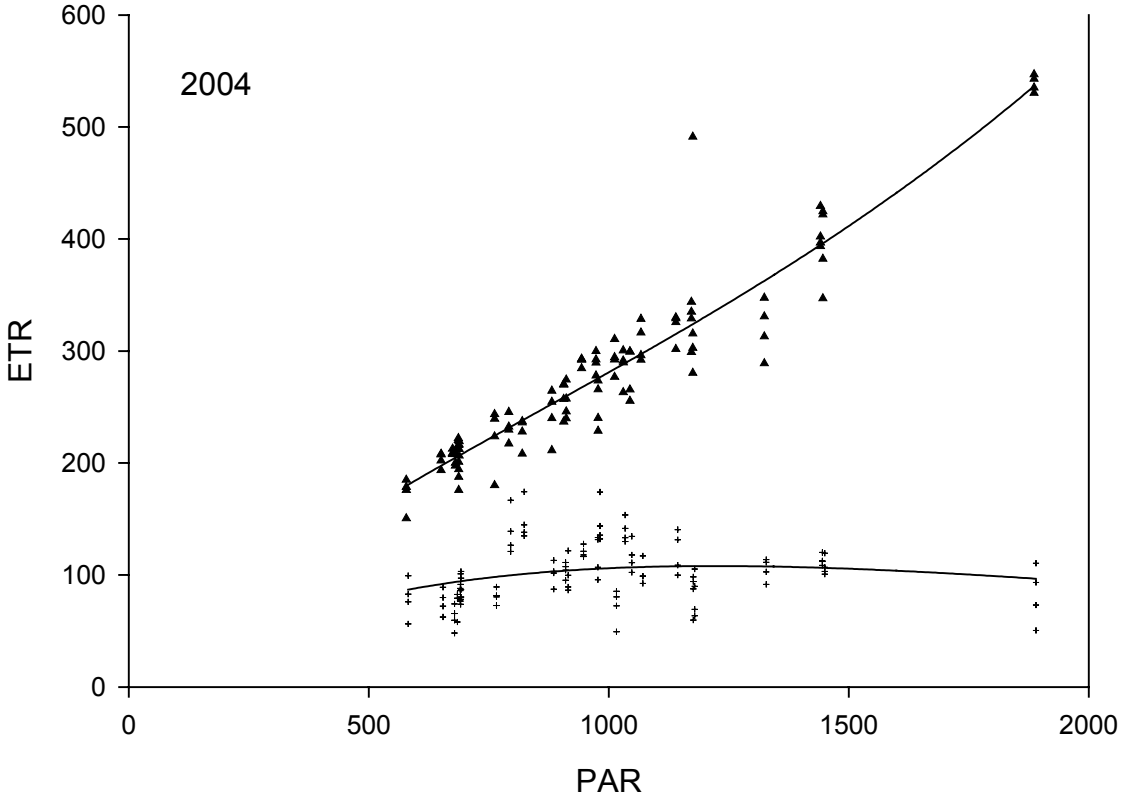
2000


2004
ETR
PAR
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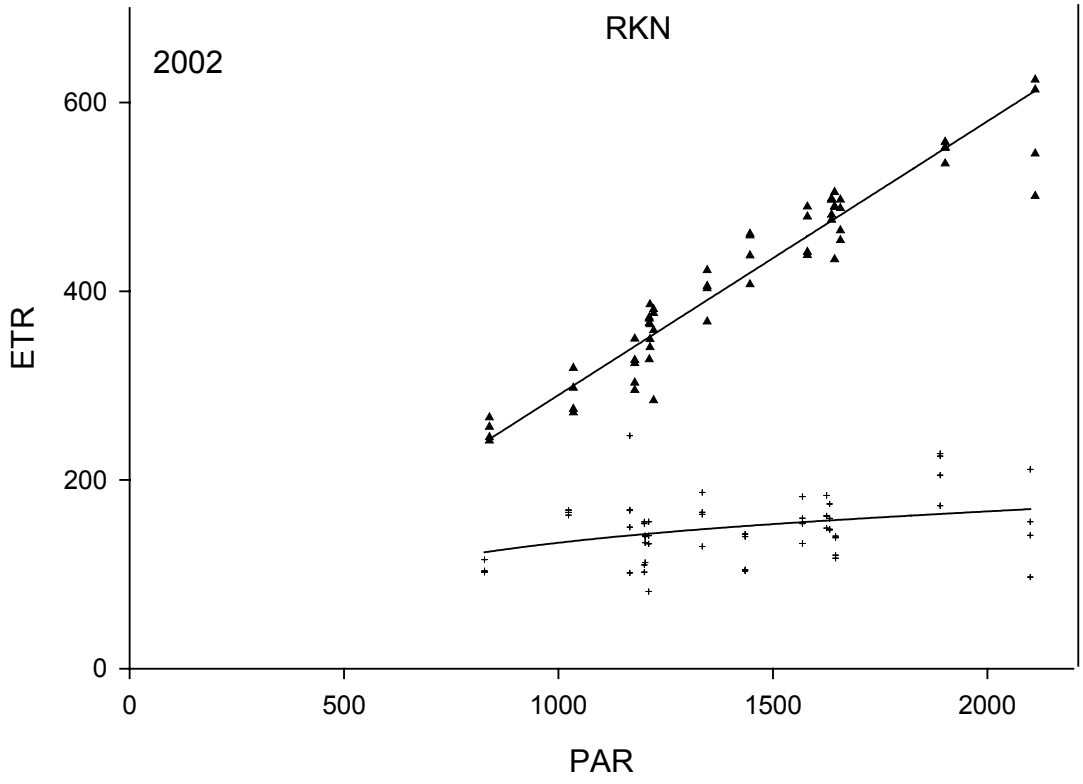
0
500
1000
1500
2000

Figure 10. Diel Yield (▲) calculated rETR vs. irradiance (PAR µmol photon $m^{-2}s^{-1}$) and Diel RLC (+) interpolated rETR vs. irradiance (PAR) for RKN in 2002 and 2004.


RKN
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ETR
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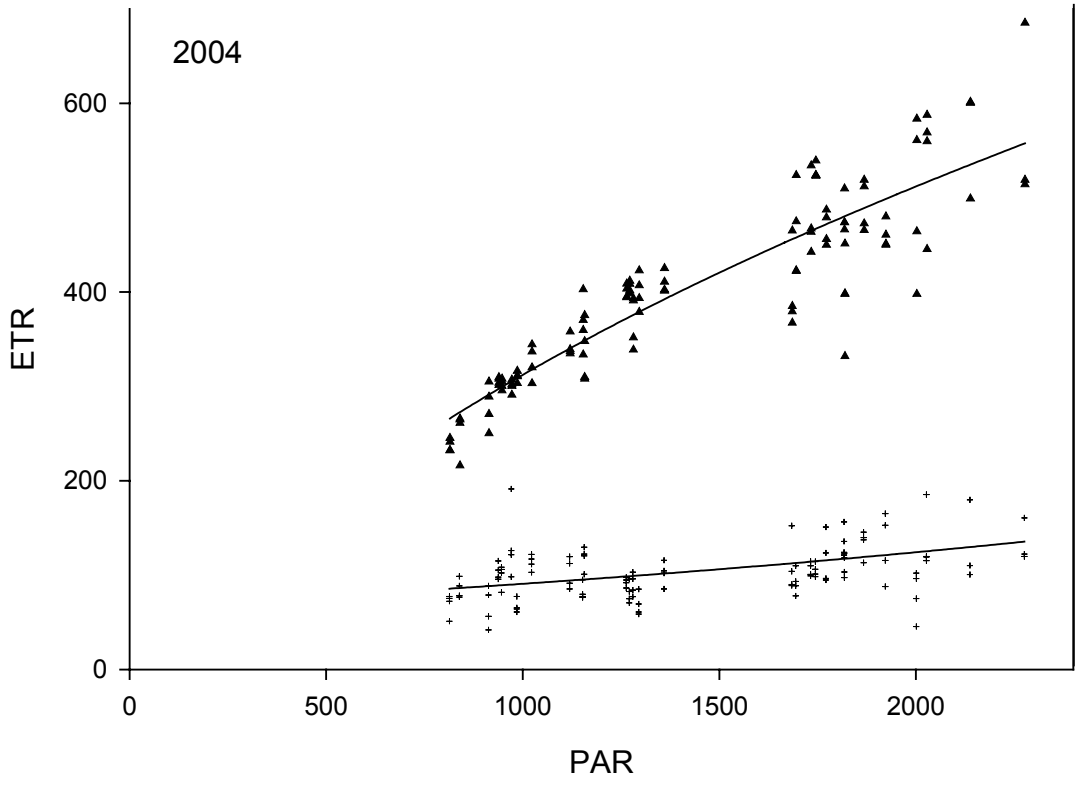
2004
ETR
PAR
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200
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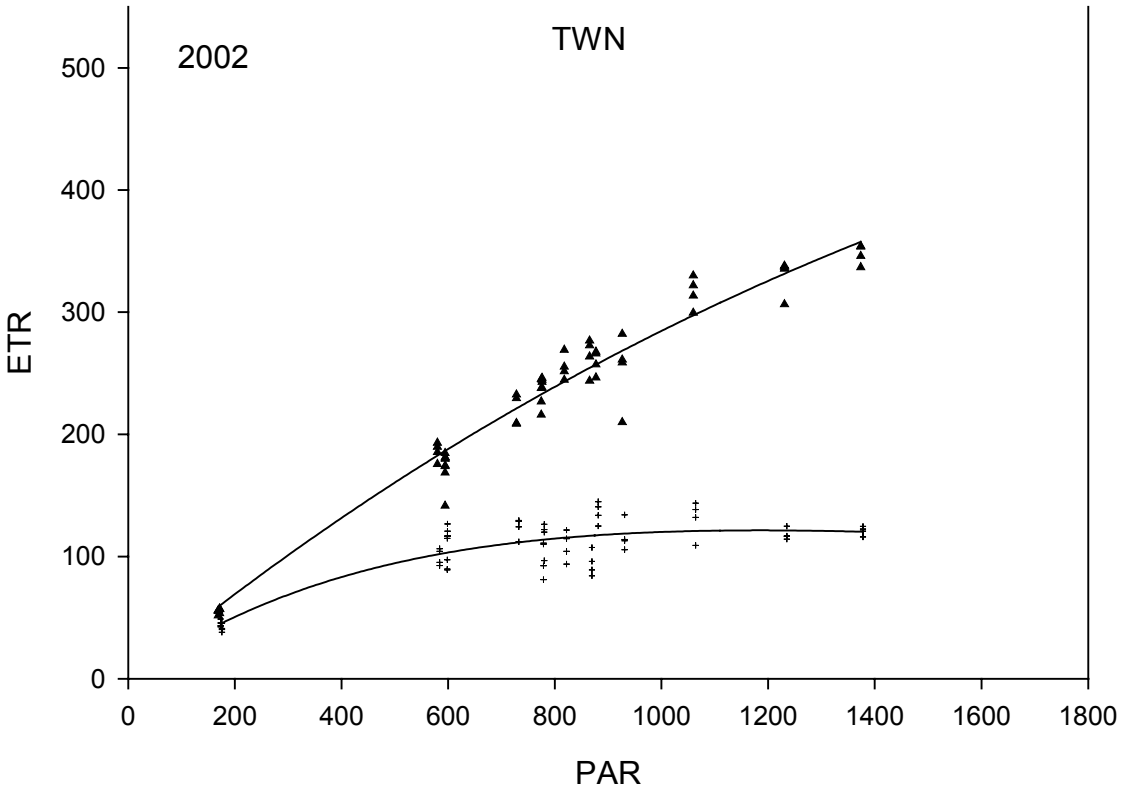
1500
2000

Figure 11. Diel Yield (▲) calculated rETR vs. irradiance (PAR µmol photon $m^{-2}s^{-1}$) and Diel RLC (+) interpolated rETR vs. irradiance (PAR) for TWN in 2002 and 2004.


2002
TWN
ETR
PAR
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100
200
300
400
500
0
200
400
600
800
1000
1200
1400
1600
1800

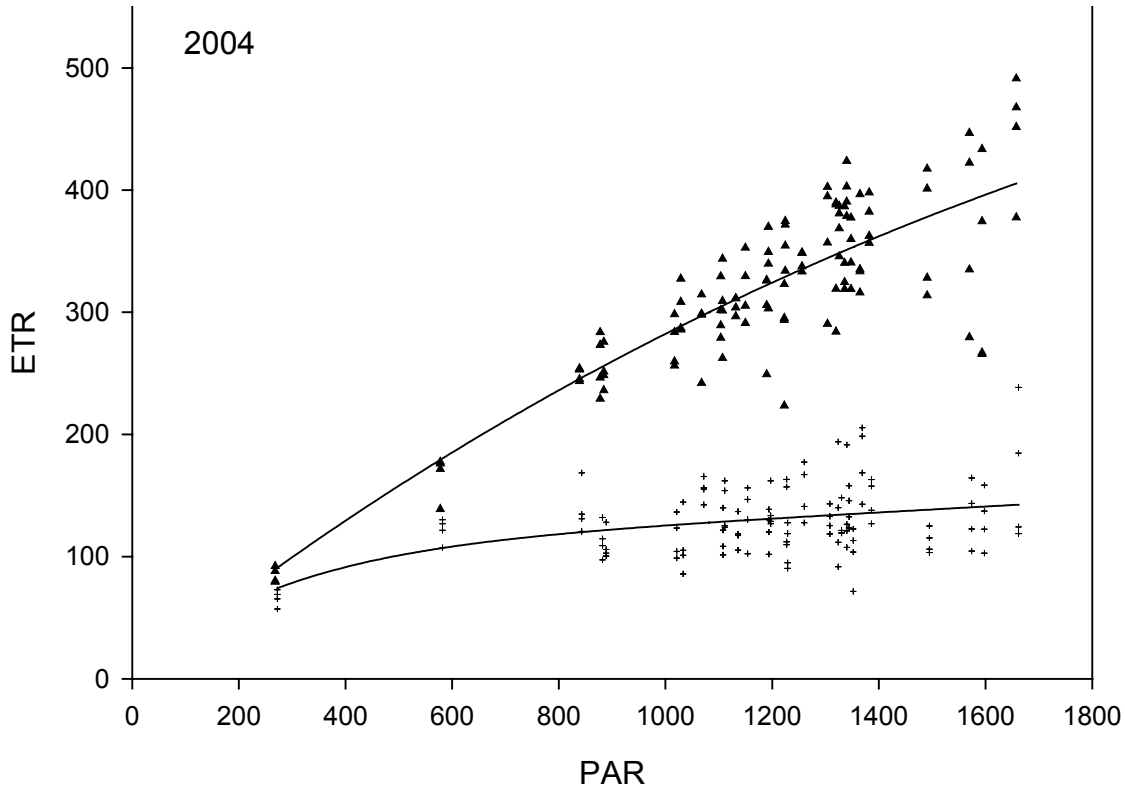
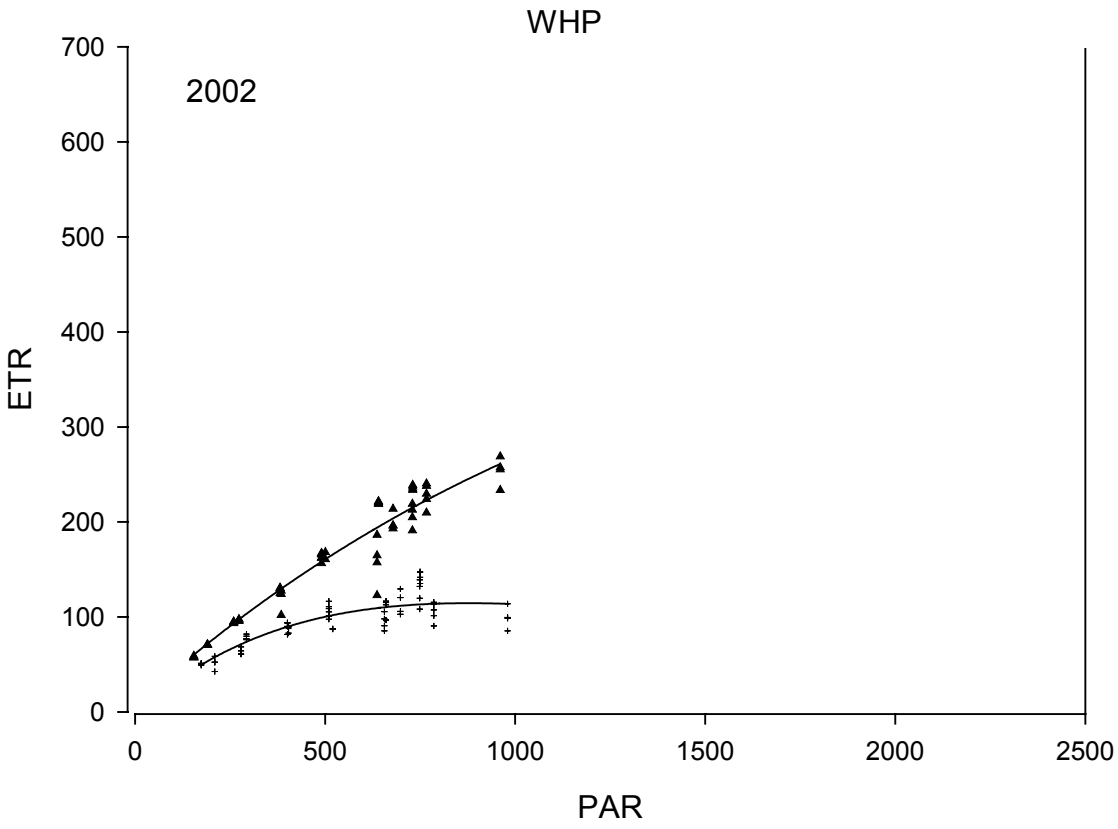
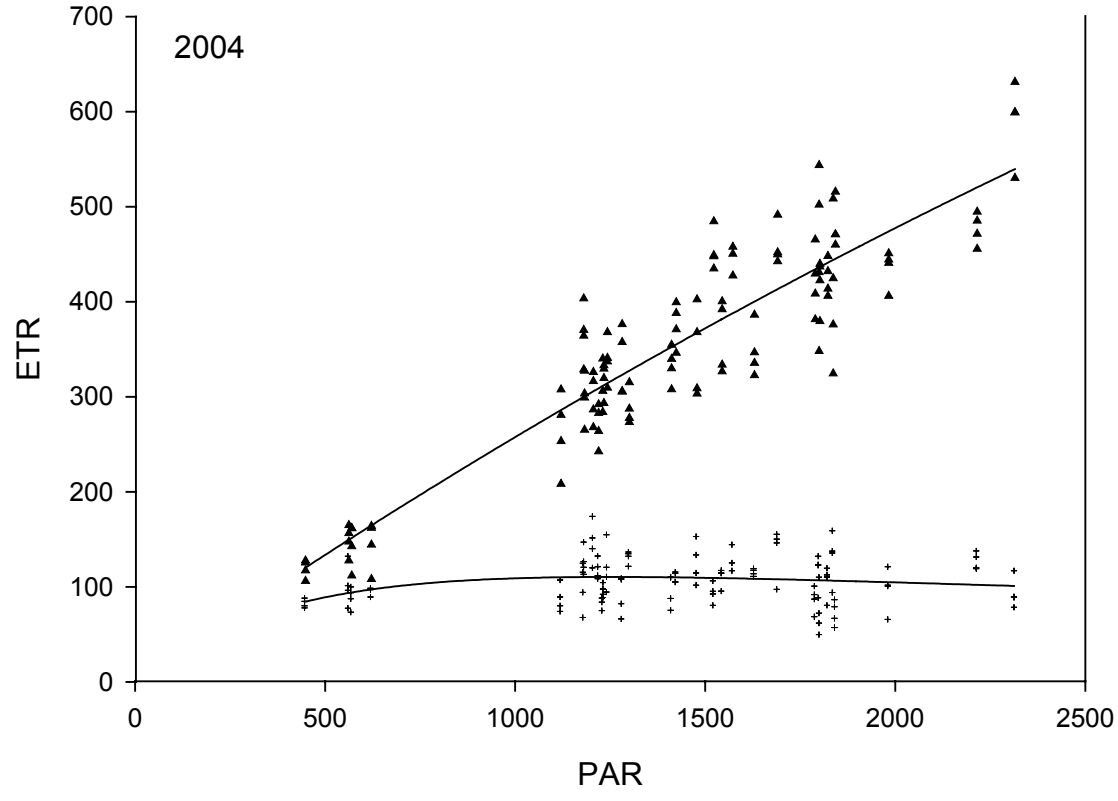

2004
ETR
PAR
0
100
200
300
400
500
0
200
400
600
800
1000
1200
1400
1600
1800

Figure 12. Diel Yield (▲) calculated rETR vs. irradiance (PAR µmol photon m^{-} s^{1}) and Diel RLC (+) interpolated rETR vs. irradiance (PAR) for WHP in 2002 and 2004.


WHP
2002
ETR
PAR
0
100
200
300
400
500
600
700
0
500
1000
1500
2000
2500


2004
ETR
PAR
0
100
200
300
400
500
600
700
0
500
1000
1500
2000
2500

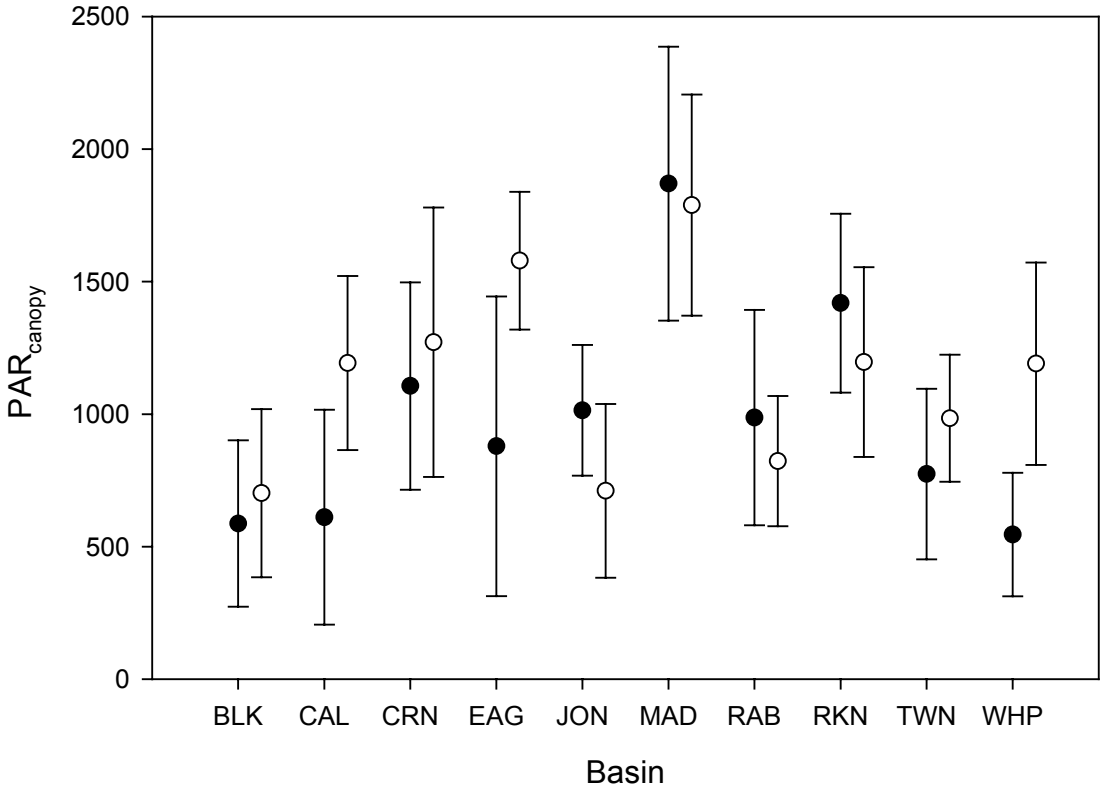


Figure 13. Mean ambient irradiance (µmol photon $m^{-2}s^{-1}$) at the seagrass canopy measured in the ten basins sampled within Florida Bay in 2002 (closed) and 2004 (open).

when the data were fitted to the double exponential decay function. Some curves exhibited a steeply increasing yet slight curvilinear appearance (BLK 04, CAL 02, CRN 02, EAG 02, MAD 04, RKN 04, TWN 02 & 04, WHP 02; Figures 3,4,5,6,8,10,11, and 12), which suggests that irradiance began to have less of an effect on the electron transport as it increased. Other Diel Yield curves showed a basic linear response (BLK 02, CAL 04, EAG 04, JON 04, MAD 02, RKN 02, WHP 04; Figures 3,4,6,7,8,10, and 12) and suggest a proportional increase in electron transport in response to increasing irradiance. In a few instances curves with a reverse asymptotic response resulted (CRN 04, JON 02, RAB 02 & 04; Figures 5,7, and 9) illustrating that as irradiance increased there was an increasingly larger effect on electron transport.

Though we were unable to statistically compare curves among basins, some informative interannual changes could be detected within basins. In some basins the two methods resulted in similar outcomes in both years (BLK, CRN, MAD, RAB, RKN, TWN, WHP; Figures 3,5,8,9,10,11, and 12), while in others there was substantial interannual variation (CAL, EAG, JON; Figures 4,6, and 7). This was mainly due to differences in irradiances when fluorescence measurements were taken. The ambient irradiance measured at the seagrass canopy varied between basins and years but generally irradiance was higher in 2004 (Figure 13). The increase in ambient irradiance in 2004 affected the P-E curves generated by the Diel RLC method. In basins where all fluorescence measurements were obtained at relatively high irradiances (CAL, EAG, and RKN, Figures 4, 6, and 10) the Diel RLC method generated P-E curves that were close to linear with zero slope.

Without measurements at lower irradiances this method was unable to generate realistic initial slopes and the resulting curves had low r^2's and unrealistically high alphas (Table 2). Also, in one basin (CRN, Figure 5) the regression for the Diel Yield method in 2004 calculated an alpha that was unrealistically high. The reason for this was not apparent by the shape of the curve, but may reflect the reverse (upward) curvature of the curve (Figure 5).

DISCUSSION

Our results indicate that the Diel Yield and Diel RLC methods suggest very different relationships between photosynthesis and irradiance for *Thalassia testudinum*. Though there was some inter-basin and inter-annual variation in the generated curves, differences between the two methods were consistent. The Diel Yield method predicted a higher photosynthetic capacity (rETRmax) than the Diel RLC method; in most basins the difference was substantial. Generally, both methods provided comparable values for photosynthetic efficiency (alpha) though there was some deviation from this trend in basins where irradiances were high throughout the entire sample period. Both methods use the same RLC data taken throughout each sample period, yet they predict very different outcomes.

Both of the diel light curve methods are based on the assumption that effective yield (Φ_{PSII}) measurements can be used to calculate a relative rate of linear electron transport (rETR). For certain seagrass species, calculated ETRs based on fluorescence measurements have a linear relationship with O_2 evolution, but for other species they only correlate at lower irradiances, with increasing discrepancies at higher irradiances (Beer et al. 1998; Beer and Bjork

2000). Also, the fraction of absorbed light must be known to accurately quantify ETRs; the default value for terrestrial plants, 0.84, was used in this study. Without knowledge of the actual amount of light being absorbed, fluorescence measurements can only be used as an approximation for electron transport (Beer et al. 1998). This is further complicated by the heterogeneity among samples taken throughout the landscape in this experiment. The assumption that light absorption is a constant for leaves growing in different microclimates in the landscape may not be true. Therefore, the information provided by these two methods is only an integrated approximation of landscape-level photosynthetic characteristics.

In P-E curves generated with the Diel Yield method, the rETR continually increased with irradiance without reaching an asymptote, resulting in unrealistic predictions of rETRmax and I_k. This method uses the first effective yield (Φ_{PSII}) measurement taken in each RLC to calculate the rETR. Because the leaf blade is covered with the dark leaf clip for a few seconds before the first yield measurement is taken, this results in a quasi dark-adapted state, which allows time for rapid re-oxidation of the primary electron acceptor (Q_A) and results in the highest effective yield measurement taken during the RLC (Ralph and Gademann 2005). Using the ambient irradiance to calculate the rETR the Diel Yield method assumes that all of the photon energy captured by chlorophyll *a*, besides that re-emitted as fluorescence, is used for photochemistry. This ignores non-photochemical quenching, which may be a major contributor of energy dissipation in high-light environments (Ralph and Gademann 2005;Marshall et al.

2000; White and Critchley 1999). Also, the increase in irradiance in the bay from sunrise to noon was disproportionately larger than the decrease in effective yield (Φ_{PSII}) measured during the mid-day. Because these relatively high effective yields are used as a multiplicand in the calculation of the rETR's, this results in disproportionably high values of rETR even though the efficiency of the system to utilize light decreases at high irradiances.

We did not take measurements of O_2 evolution in this study, but O_2 based values of photosynthetic capacity reported for *T. testudinum* in Florida Bay range from 171-256 μg O_2 ^{2} $g^{-1}min^{-1}$ with saturation irradiances (I_k) ranging from 357-438 μE $m^{-2}s^{-1}$ (Fourqurean and Zieman 1991). In comparison, calculations of I_k based on fluorescence measurements for the Diel Yield method were generally higher, ranging from 297-4813 μE $m^{-2}s^{-1}$ (except for CRN in 2004, Table 2). Because of the frequent unrealistically high I_k values, we believe that the Diel Yield method does not provide a good indication of photosynthetic capacity. When comparing the Diel Yield curves to curves calculated from O_2 evolution, Longstaff et al. (2002) found that they only correlated at lower irradiances. The Diel Yield based rETR curve continued to increase as irradiance rose while the O_2 based curve tended toward asymptote. This indicated that an increased number of electrons were flowing through the photosystems for every O_2 evolved (Longstaff et al. 2002). This could be due to increased photorespiration (Beer et al. 1998) or an increase in alternative forms of energy dissipation, such as non-photochemical quenching (Longstaff et al. 2002). At higher irradiances similar

amounts of fluorescence may be detected without increases in photosynthesis because of increased non-photochemical quenching.

The Diel RLC method produces curves indicating different rETR versus PAR relationships, and resulted in P-E curves that did reach an asymptote and provided reasonable estimations of rETRmax and I_K. With this method, the RLCs produced from the applied actinic irradiances were used to interpolate the rETR at the measured ambient irradiance taken at the time of the RLC. As actinic irradiance increases during a RLC, photochemical quenching decreases and non-photochemical quenching increases, due to an accumulation of electrons on the PSII acceptor side (Schreiber 2004 cited in Ralph and Gademann 2005; White and Critchley 1999). This results in a reduction of fluorescence and photochemical quenching as irradiance increases. Therefore, the interpolated rETR from this method is generated from actual measured responses of fluorescence to various light levels, which incorporates changes in photochemical and non-photochemical quenching. As a result, we believe that it is a more accurate representation of *T. testudinum's* photosynthetic responses to irradiance.

In this study, the fluorescence-based diel light curves generated from the two methods yielded different trends from those reported by Longstaff et al. (2002). They reported that the two methods provided comparable rETRmax estimates, but differing alphas, even though their curves resembled ours. A major difference between our experimental designs was the ambient irradiance in which the fluorescence measurements were taken. They reported a daily

maximum irradiance of 400 µmol photons $m^{-2}s^{-1}$, while we measured irradiances that were up to six times as high. Because of the high light conditions (>I_k all day) at some of the basins in Florida Bay, relatively frequent miscalculations of photosynthetic efficiency resulted. The ambient irradiance is used in the calculations of rETR and P-E curves for both methods. In situations where irradiance was always high this resulted in a relative absence of data points for the initial part of the curves. As a result, both methods had difficulties predicting realistic slopes (alpha). Even though the Diel RLC method was able to more realistically predict rETRmax and I_k, this method had the most difficulty predicting alpha. With this method the rETR is interpolated from RLCs that do reach saturation and level out. Therefore, the interpolated rETR at high irradiances coincide with the saturated part of the curve and when all irradiances are all >I_k, nearly flat (slope = 0) lines result. This was reflected in the low r^2 of the double exponential decay regressions between irradiance and rETR using the Diel RLC method. It was largely because of the near linearity of the response of rETR and irradiance with the Diel Yield method that a reasonable slope (alpha) could be mathematically calculated from this model, in most instances.

Conclusion

Though both light-curve methods incorporated the entire spatial and temporal scale of sampling, neither method was able to provide consistent estimates of alpha, rETRmax or I_k to allow unbiased *in situ* assessments of the photophysiology of the seagrass, *T. testudinum*, in Florida Bay. Though statistically (as determined by r^2 of the regressions) the Diel Yield method was

found to be a better predictor of the relationship between rETR and irradiance, this method frequently produced unrealistic predictions of photosynthesis at high irradiances. The Diel RLC method produced more reasonable predictions of photosynthetic capacity, but it was unable to predict photosynthetic efficiency when ambient irradiances were continuously high throughout the day ($>I_k$). In order to overcome this we suggest using the original data from the RLCs, which provide an actual measured photosynthetic response to lower light levels. Since it has been determined that the initial slope of RLC's do exhibit diurnal variation (White and Critchley 1999; Ch 1), taking the mean of the slopes taken throughout the day would give a reasonable approximation of the photosynthetic response of the seagrass to the ambient range of light levels. We believe that with some further calculations to estimate a realistic slope, the Diel RLC method can be used to approximate the relationship between irradiance and electron transport.

Both methods investigated in this study use data generated from RLCs, and it has been shown that actinic irradiances administered during RLCs affect leaves with varying irradiance histories differently (White and Critchley 1999 and Ch 1). As a result, diurnal variations of ambient irradiance, photochemical quenching and non-photochemical quenching do affect estimates of electron transport rates. Therefore, the Diel RLC method does not negate diurnal variation but it does produce a curve that incorporates the changing ambient light environment. There are clearly other mechanisms of energy dissipation that can result in unreliable fluorescence measurements at higher irradiances. Further research on the

effects of photochemical and non-photochemical quenching of fluorescence in high light situations is warranted.

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